

Functional Organization of Crayfish Abdominal Ganglia: II. Sensory Afferents and Extensor Motor Neurons

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Abstract:

Abdominal ganglia of crayfish contain identifiable neuropils, commissures, longitudinal tracts, and vertical tracts. To determine the functional significance of this ganglionic framework, we backfilled the following types of neurons with cobalt chloride: sensory hair afferents, slow and fast extensor motor neurons, the segmental stretch receptor neurons, and their inhibitory accessory cells. After the cobalt ions were precipitated and intensified, we studied the central projections of the filled neurons within the ganglionic structures. All of the axons of these neurons exit or enter each of the first five abdominal ganglia through the second pair of nerves.

Our description of the central projections of the hair afferents is the first in the literature. These afferents innervate the large ventral horseshoe neuropil (HN) in the core of each ganglion. This neuropil is homologous to the insect ventral association centers, which also process sensory information. Furthermore, we discovered that some of the crayfish afferents innervate glomeruli within the HN.

The slow and fast extensor motor neurons, the stretch receptor neurons, and the accessory cells branch mostly in the dorsal part of the ganglion. We reinterpret previous identifications of the extensor neurons that were based largely on soma position. Together with our previous descriptions of the flexor motor neurons, these results allow us to relate both rapid tail-flips and slower postural movements to the structure of the segmental ganglia. Key words: cobalt backfills, commissures, glomerulus, neuropil, tracts

Abbreviations

a#	anterior slow extensor neurons
A	anterior fast extensor neuron
Acc-#	accessory neurons 1-4
AVC	anterior ventral commissure
C#	contralateral fast extensor motor neurons
CM	cutaneous mechanoreceptors
DC#	dorsal commissures (numbered 1-7)
DIT	dorsal intermediate tract
DLT	dorsal lateral tract
DMT	dorsal median tract
e1	slow extensor motor neuron with contralateral soma
HN	horseshoe neuropil
LDT	lateral dorsal tract
LG	lateral giant axon
LN	lateral neuropil
LVT	lateral ventral tract
MDT	median dorsal tract
MG	medial giant axon
MRO	muscle receptor organ
MVT	median ventral tract
N#	nerve 1, 2, or 3
n.p.m.	nerve to profundis muscles
n.r.m.	nerve to muscle receptor organ
p#	posterior slow extensor neurons
P#	posterior fast extensor neurons
PVC	posterior ventral commissure
SB#	sensory branches of nerve 2
SR	stretch receptor
TN	tract neuropil
VAC	ventral association center
VIT	ventral intermediate tract
VLT	ventral lateral tract
VLT _i	inner ventral lateral tract
VLT _o	outer ventral lateral tract
VMT	ventral median tract

Article:

A common feature among many invertebrate phyla is the development of a brain as the product of fusion of several anterior ganglia. In brains, distinct lobes, or neuropils, can be identified and correlated with specific behavioral or integrative functions (Bullock and Horridge, '65; Sandeman and Luff, '73; Ernst et al., '77; Ernst and Boeckh, '83). In arthropods, the segmental ganglia also contain identifiable neuropils (Kendig, '67; Gregory, '74; Tyrer and Gregory, '82; Skinner, '85a,b), as well as recognizable commissures and tracts (Altman and Kien, '79; Altman, '81; Braunig et al., '81; Romer, '83; Johnson and Murphey, '85; Levine et al., '85; Murphey, '85; Paul and Mulloney, '85; Skinner, '85a; Wohlers and Huber, '85). The hypothesis that different neuropils are responsible for the different motor and sensory roles played by a ganglion was based on analyses of insect ganglia (Altman and Kien, '79; Altman, '81). Since then, the structure of the abdominal ganglia of a crayfish has been described in similar detail (Skinner, '85a,b). To determine if this hypothesis applies to crustaceans and to understand how the architecture of these ganglia relates to their functional roles, we are making a systematic study of the populations of motor and sensory neurons whose central projections lie within these ganglia. In the preceding paper we described the pathways taken by the fast and slow flexor motor neurons within the various ganglionic structures (Leise et al., '86). These descriptions supported the application of Altman's hypothesis to crustacean nervous systems.

In this paper we report on the pathways used by neurons whose axons lie in the second pair of ganglionic nerves (N2). The anterior branch of this nerve contains sensory afferents from hairs on the body wall; this branch divides several times so that smaller branches innervate distinct fields of hairs on the exoskeleton (Hughes and Wiersma, '60; Wine and Hagiwara, '77). The central projections of the hair afferents have not previously been described. Efferents to the slow and fast extensor muscles lie in the posterior branch of N2 along with components of the muscle receptor organs (MROs): the two stretch receptors and their four inhibitory accessory cells (Alexandrowicz, '51; Eckert, '61; Wine and Hagiwara, '77). This posterior limb of N2 separates into two smaller branches: the n.p.m. innervates the fast extensor muscles, and the n.r.m. innervates the slow extensor muscles, the MROs, and a row of hairs on the posterior margin of the dorsal exoskeleton (Parnas and Atwood, '66; Triestman and Remler, '75). The three types of efferents mentioned are active in several different behaviors, including tail-flip escape responses and backward swimming (Wine, '77a,b, '84; Wine and Hagiwara, '77) and postural extension (Parnas and Atwood, '66; Miall and Larimer, '82a,b; Edwards, '84). The identification of these motor neurons has been attempted several times (Triestman and Remler, '75; Wine and Hagiwara, '77), but no description exists of the locations of their central projections within the ganglionic structures.

The results presented here support Altman's ('81) hypothesis that different neuropils play distinctive roles in the generation of complex behaviors. They also allow us: (1) to reevaluate previous identifications of fast and slow extensor motor neurons (Triestman and Remler, '75; Wine and Hagiwara, '77), (2) to identify sensory afferents as the incoming and potentially presynaptic elements in the glomeruli within the horseshoe neuropil, (3) to confirm the identification of the accessory cells of the muscle receptor organs, and (4) to discover the position of all of these neurons within the ganglionic structures. We also discuss the routes of these neurons in terms of their known integrative activities.

METHODS

Adult crayfish, *Pacifastacus leniusculus*, were obtained from California Valley Fish. Co., Hood, CA, or from Cliff's Marina, Freeport, CA. They were maintained in many 10-gallon aquaria with approximately six individuals per aquarium. Gravid females were isolated in separate aquaria to increase the survival rate of juveniles. The female was removed from the tank after the juveniles had ceased to cling to her. Added to these tanks was the aquatic plant *Anacharis sp.*, which the juveniles fed on for many weeks. After several months, juveniles were fed Trout Chow (Purina, Inc.) pellets, one pellet per animal, three times per week.

The neuroanatomy of abdominal ganglion G4 was described by Skinner ('85a,b) for *Procambarus clarkii*. In general, her results apply to *Pacifastacus leniusculus*. Minor differences between ganglia in the two species were previously discussed (Leise et al., '86) and are reviewed as necessary in the Results section.

Cobalt backfills of efferent neurons and stretch receptors

Cobalt backfills (Iles and Mulloney, '71) were made from various branches of the second nerve (N2) in abdominal ganglia 2-5 (G2-G5) in animals of both sexes. All extensor muscle backfills were done on small animals, 2-4 cm long (measured from end of telson to tip of rostrum). In such animals, successful fills of the stretch receptor (SR) axons spanned the entire abdominal nerve cord. We also filled extensor neurons in several large animals, about 8 cm long, for comparison. A total of 23 ganglia contained fills of slow extensor motor neurons, SR axons, and accessory cells. Fourteen ganglia contained filled fast extensor motor neurons. The fast extensor results presented here are derived from the six best preparations.

Backfills of fast and slow extensor motor neurons, SR axons, and accessory neurons were made on in vivo preparations. Dissections were made on animals whose cephalothorax and walking legs were immobilized with rubber bands. In all dissections only a small piece of the dorsal exoskeleton and underlying epidermis was removed. The chosen nerve was then teased from the muscles and connective tissue through this window. A vaseline well was constructed on the nearby exoskeleton, a small drop of 0.2 M CoCl_2 was put on top of the well, the nerve was placed in the cobalt solution, the nerve end was again cut, and the well covered with vaseline. Fast extensors were filled by dissecting out the n.p.m. (nerve to profundis muscles, Triestman and Remler, '75) and cutting it near its insertion on the muscles. Slow extensor motor neurons, stretch receptor (SR) axons, and the accessory neurons were filled by dissecting out the n.r.m. (nerve to muscle receptor organ MRO) and cutting it near its insertion point. Each animal was left in its dissecting dish in a small amount of crayfish saline (Paul and Mulloney, '85), in a 10°C cold room for 24 hours. At the end of this time, the nerve cord was dissected from the animal, pinned out in a Sylgard-lined Petri dish in fresh, cold saline, and the cobalt precipitated as cobalt sulfide with $(\text{NH}_4)_2\text{S}$. This final dissection and the precipitation procedure, subsequent fixation, and sectioning methods were carried out as previously described (Leise et al., '86).

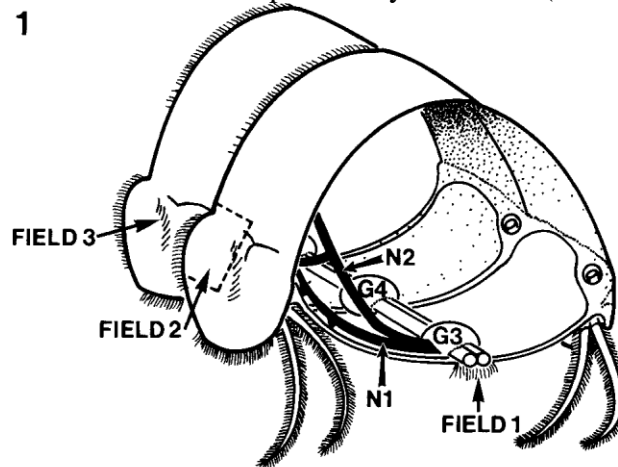


Fig. 1. Diagram of three sensory fields innervated by neurons exiting the right half of G3 in the third and fourth abdominal segments (after Hughes and Wiersma, '60; Wine and Hagiwara, '77).

Cobalt backfills of hair afferents

Two types of fills were made from sensory hair afferents. In in vivo preparations, animals, 8-10 cm long, were restrained as described. Moist paper towels were wrapped around the cephalothorax. A small piece of exoskeleton lateral to the ventral midline and including one sternal rib was removed. The underlying square of epidermis was cut away from the surrounding tissue leaving its branch of nerve 2 (sensory branch 1, SB1) attached. SB1 was dissected free from any connective tissue. The filling procedure was the same as that used for extensor backfills. When manipulating the preparation, only the square of epidermis was touched, to avoid damage to the nerve. The epidermis was cut off after the cobalt chloride was applied. The pool of cobalt and the wound were covered with vaseline and the animal was placed in a small amount of fresh tapwater and left in the 10°C cold room for 14-26 hours. Again, the final dissection and subsequent processing methods were described in Leise et al. ('86).

We obtained successful fills of sensory hair afferents in 37 ganglia. In addition to fills of SB1, we also filled the entire anterior branch of N2 in vitro. To do this, nerve cords were dissected from animals as previously

described (Leise et al., '86), pinned out in a Sylgard-lined dish, and fills made of the anterior branch of one or more N2s. A 0.4 M sucrose well was used proximal to the cobalt well in these fills (Mulloney, '73; Leise et al., '86). The best in vitro fills were obtained from nerve cords exposed to cobalt chloride for 4 hours. Longer filling times yielded ganglia in which the axons in the nerve and anterior connectives were filled, but in which the ganglionic core was unmarked. Sensory afferents of crayfish are known to degenerate much more rapidly than motor neurons when cut off from their somata (Nordlander and Singer, '74). During the longer filling times (5-24 hours), we suspect that the central arborizations of these cells were dying and releasing the cobalt into the extracellular fluid.

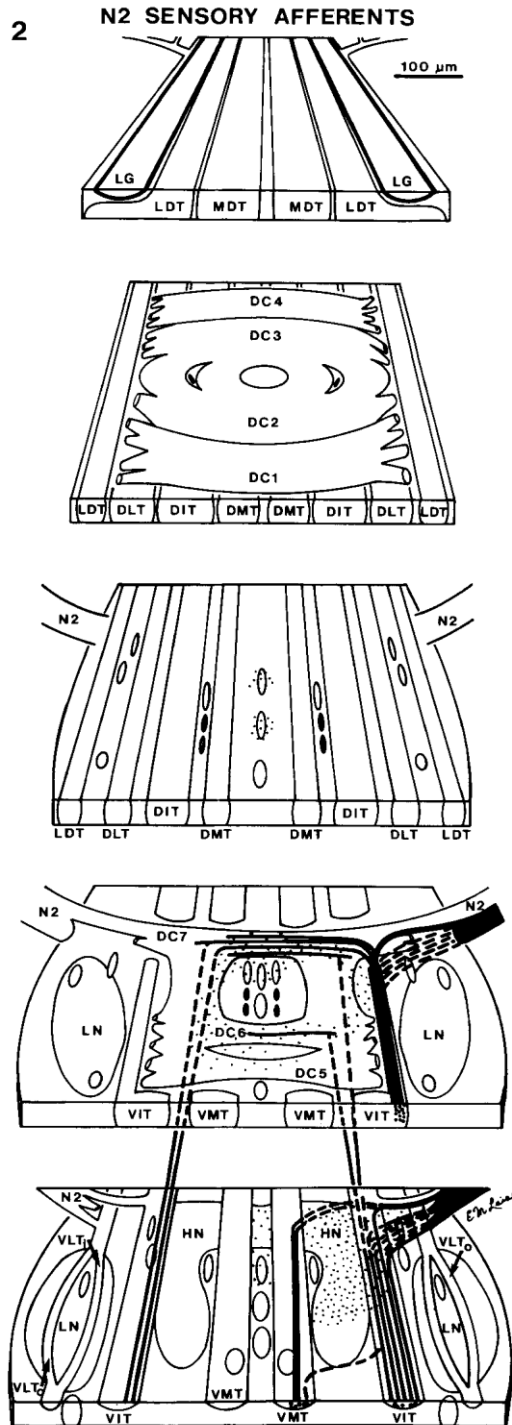


Fig. 2. Main neurites (solid and dashed lines) and regions containing branches (stippling) of sensory afferents from exoskeleton hairs, diagrammed in five major levels of an abdominal ganglion (cf. Fig. 24, Leise et al., '86). Each section is a slab of tissue approximately 50 μ m thick. Solid lines represent those parts of the neurites that lie in or above the plane of a slab; dashed lines represent the parts of the neurites lying below a slab. View is toward the posterior.

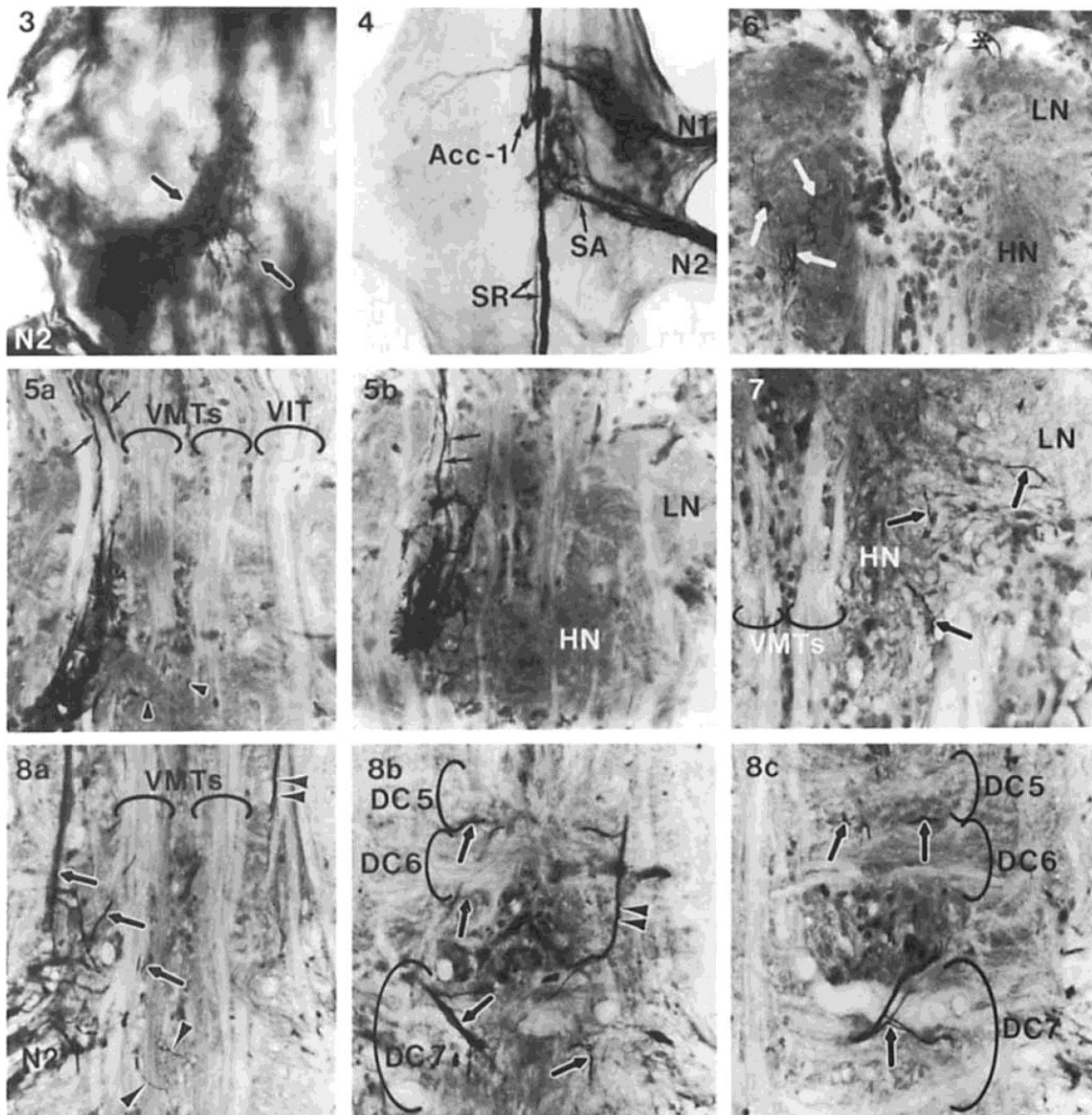


Fig. 3. G4, ventral view of right hemiganglion, sensory hair afferents filled from the anterior branch of N2. Tangle of branches (between arrows) in HN indicates an abundance of filled axons. No descending axons occur in the connectives. $\times 110$.

Fig. 4. Dorsal view, G4. Concurrent fills of sensory hair afferents (SA) from the anterior branch of N2 and swimmeret motor neurons from N1. Afferents branch profusely in the HN. Stretch receptor (SR) axons from G3 occur in the ipsilateral dorsal median tract. The axon of Acc-1 from G3 lies next to the SR axons for part of its travel in the connective. Neurons in this ganglion were filled for 26 hrs. $\times 70$.

Fig. 5. a and b. G4 (cf. Fig. 3), serial longitudinal sections through the HN and third longitudinal tract layer. Sensory afferents branch profusely in the ipsilateral HN and send axons rostrally in the VIT (arrows). A few branches also occur in the curve of the HN (arrowheads). $\times 120$.

Fig. 6. Longitudinal section through the bases of the HN and LNs in G4 (cf. Fig. 3). Sensory afferents (arrows) branch only in the ipsilateral HN. Asterisk is on a sectioning artifact. $\times 145$.

Fig. 7. G2, longitudinal section through the base of the ipsilateral HN and LN. Branches of sensory afferents (arrows) occur in both neuropils. $\times 135$.

Fig. 8. G4, serial longitudinal sections of sensory hair afferent branches (arrows) in the third longitudinal tract layer (a) and second commissure layer (DC5-DC7) (b,c) (cf. Fig. 9b). Branches occur in the curve of the HN (arrowheads in a). Rostral axons extend ipsilaterally (left arrow in a) and contralaterally (double arrowheads in a and b) in the VITs. $\times 125$.

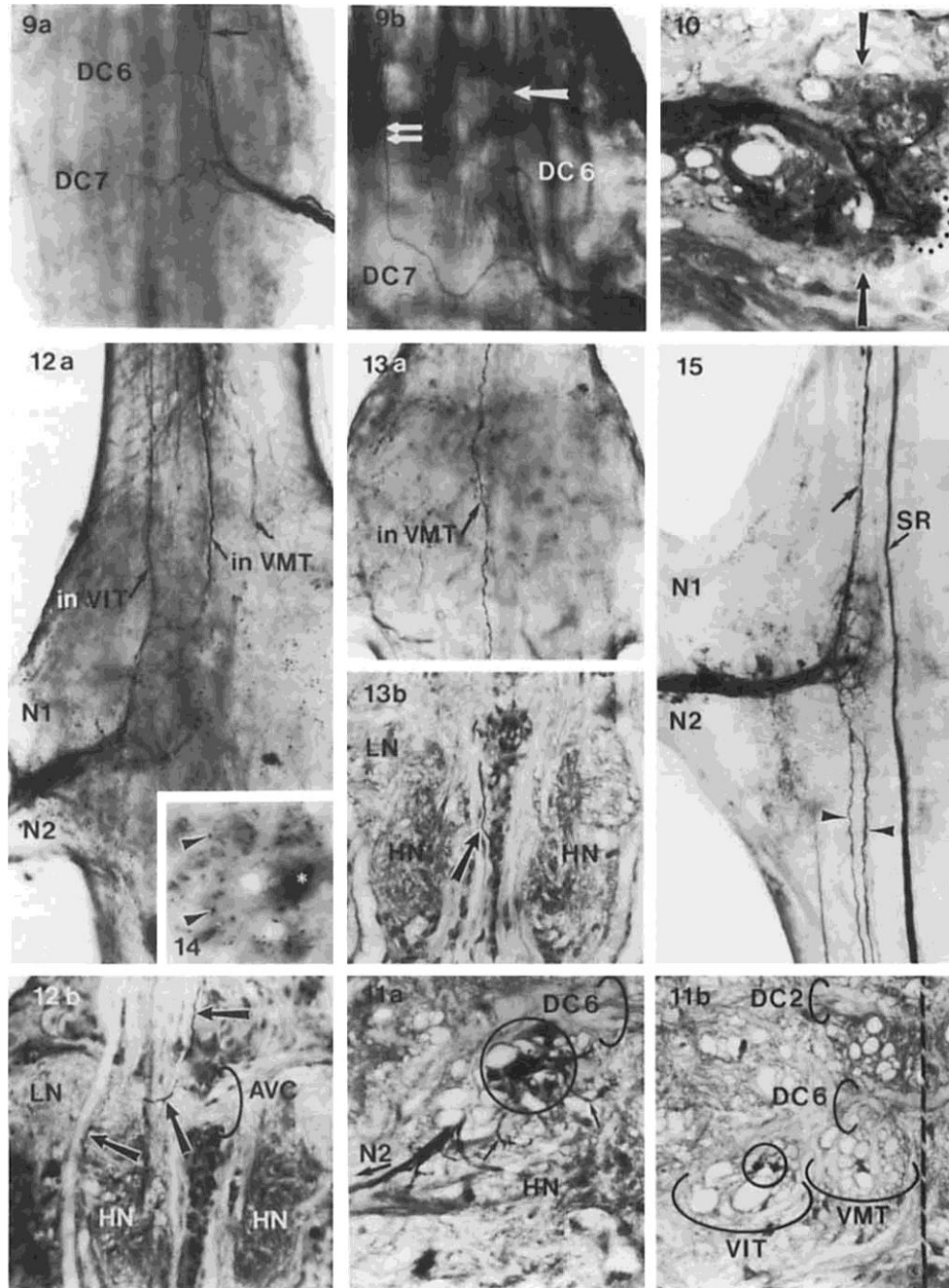
All measurements were made from sectioned material. Measurements of branch lengths of stretch receptor (SR) axons were made from four well-filled SR axons in G2-G5. Serial thick (15 μm) sections were drawn with the aid of a camera lucida. Branch lengths were digitized from the drawings using a digitizing tablet linked to a microcomputer.

RESULTS

Paths of the primary sensory afferents from exoskeleton hairs

The N2 primary sensory afferents that are not stretch receptors arise from exoskeleton hairs in at least four different fields. The anterior branch of N2 divides into several sensory branches: sensory branch 1 (SB1)

innervates a field of hairs on the midline of the ventral exoskeleton rib in the segment containing the ganglion of origin (Fig. 1). This branch may also contain axons from the cutaneous mechanoreceptors that innervate the hypodermis under the soft ventral cuticle (Pabst and Kennedy, '67). SB2 innervates hairs on a rectangular area of the ipsilateral pleural plate in the same segment (Wine and Hagiwara, '77), and SB3 innervates an arc of hairs in the next caudal pleural plate (Wine and Hagiwara, '77). Additional hairs on the tergum (dorsal exoskeleton plate) of this posterior segment are also innervated by N2 (Hughes and Wiersma, '60). We made axonal fills of both the entire anterior branch of N2 and of SB1 alone.



Figures 9-15

Fig. 9. a. G3. b. G4. Wholemounds of filled sensory hair afferents, dorsal views. Filled branches cross to the contralateral hemiganglion in two commissures, DC6 and DC7. Axons extend rostrally in the ipsilateral (arrow) and contralateral (double arrows in b) VITs. $\times 75, 90$.

Fig. 10. G5. Transverse section through the HN (between arrows) in left hemiganglion. One glomerulus (partially circled) is innervated by a bundle of several sensory hair afferents.

Fig. 11. a and b. Transverse sections through the left hemiganglion of G2 and G5, respectively. a. Six or seven sensory hair axons (circled) in the VIT send branches (arrows) into the adjacent HN. b. Two sensory axons circled lie in the VIT. Vertical dashed line indicates ganglionic midline. $\times 260$.

Fig. 12. a. G3, ventral view of afferents from SB1. Rostral axons lie in the ipsilateral VMT and VIT. $\times 95$. b. Longitudinal section through the HN and AVC of this ganglion. Medial axon (arrows) crosses from the VIT to the VMT in the AVC. $\times 110$.

Fig. 13. G2, rostral axon from G3 sensory hair afferent. Wholemound (a) and longitudinal section through HN (b). Axon (arrow) is unbranched. $\times 75, 135$.

Fig. 14. Transverse section through N2 containing one large filled sensory axon (asterisk) and 30-40 small ($< 1 \mu\text{m}$) axons (arrowheads). $\times 570$.

Fig. 15. Dorsal view, G2. Sensory hair afferents from anterior branch of N2 travel rostrally (arrow). Cobalt chloride was applied to this N2 for 26 hours. This resulted in leakage of cobalt ions, which were picked up by descending axons (arrowheads). They are not connected to any sensory afferent. Stretch receptor (SR) axons arise from fills in posterior ganglia. $\times 65$.

The central projections of the SB1 afferents follow paths similar to those we obtained from fills of the entire anterior branch of N2. Thus, here we describe fills of entire anterior branches and only refer to SB1 afferents where they differ from the basic pattern.

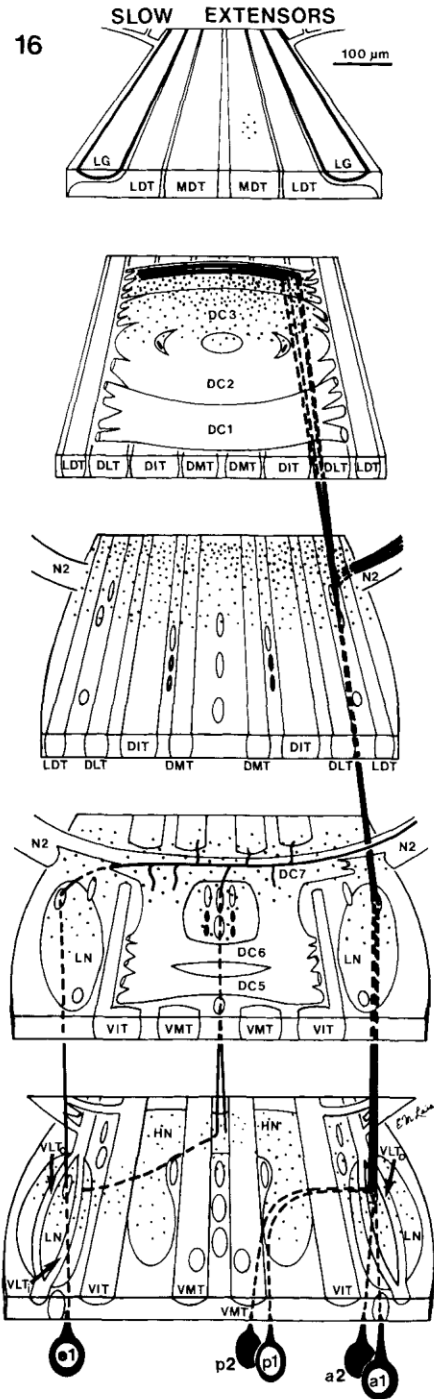
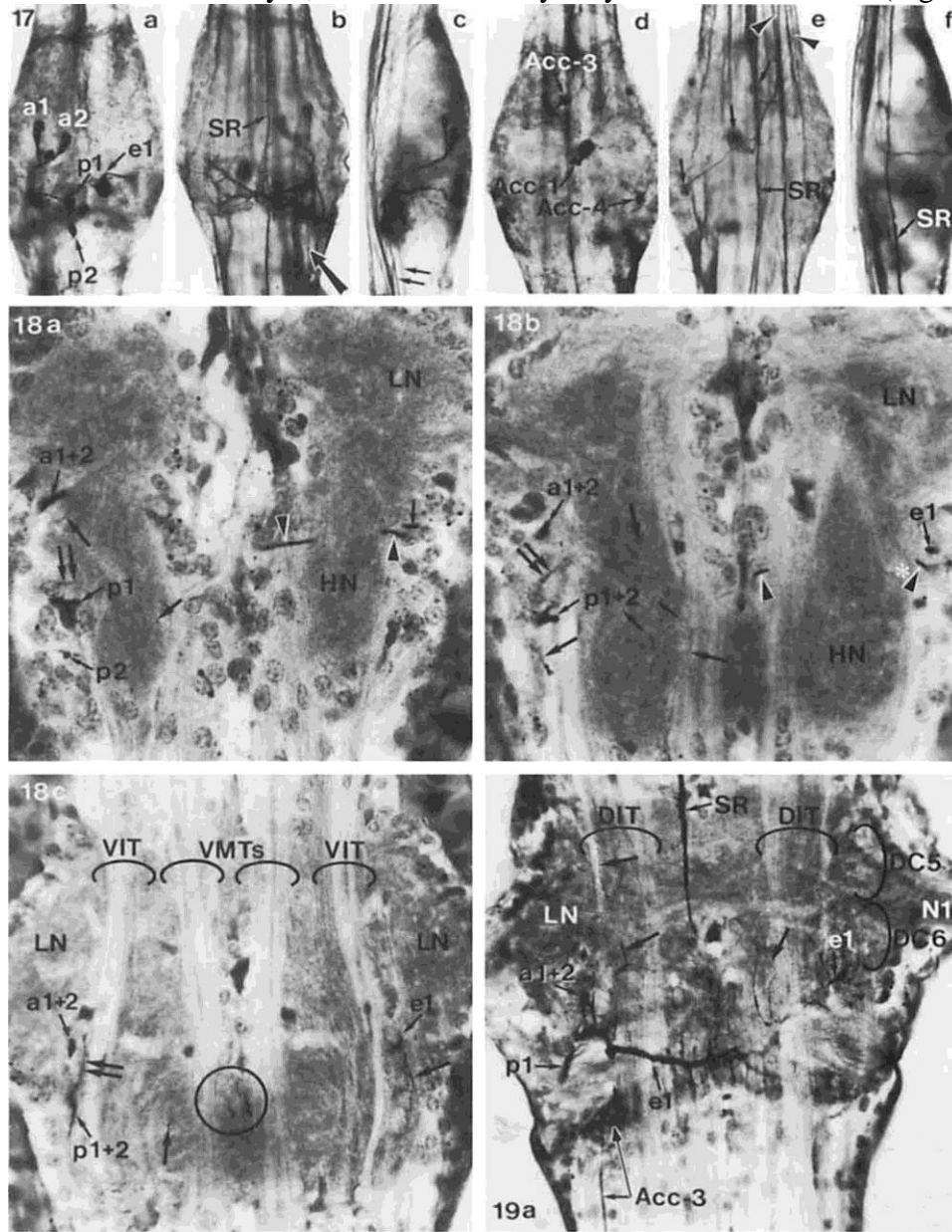


Fig. 16. Diagram of main neurites (solid and dashed lines) and regions of dendritic branching (stippling) of slow extensor motor neurons in five levels of a stylized ganglion.

The somata of the N2 sensory hair afferents are all peripheral. The main target for their central branches is the horseshoe neuropil (HN, Fig. 2). In whole mounts, these branches are seen as tufts of short processes just at the point where the axons turn rostrally (Figs. 3, 4). Branches occur throughout the central part of the ipsilateral arm of the HN (Fig. 5) and extend into the base of the HN where it is continuous with the lateral neuropils (LNs) (Fig. 6). However, the axonal branches do not fill this lower part of the HN arm as they do the more dorsal part. In a few ganglia, branches project into the LNs (Fig. 7). Branches also extend into the curve of the HN (Figs. 5, 8a) but not into its contralateral arm. Dorsally, branches reach the second commissure layer (Fig. 8) and end in the lower part of the second longitudinal tract layer (Fig. 2). In 11 ganglia, branches 2-6 μm in

diameter crossed to the contralateral hemiganglion in dorsal commissures 6 and 7 (DC6, DC7) (Figs. 8, 9). Several ganglia also contain filled branches that reach DC5. In summary, axonal branches occur ipsilaterally, in both the HN and second commissure layer, but contralaterally only in the commissures (Fig. 2).



Figures 17-19

Fig. 17. *a-c*. G3, five filled slow extensor motor neurons, ventral, dorsal, and side views. Filled nerve was to the left in *a*, to the right in *b*. One stretch receptor axon (SR) filled and projected to G6 and past G1. *d-f*. G4, ventral, dorsal, and side views of three accessory neurons, Acc-1, Acc-3, and Acc-4, filled concurrently with the slow extensors in G3. Filled nerve was to the left in (*d*), to the right in (*e*). Arrows in (*b*) and (*c*), accessory cell axons. Arrows in *e* point to accessory somata. Only Acc-3 and Acc-1 are visible in *f*. Arrowheads in (*e*) point to their axons. $\times 85$.

Fig. 18. G3 (cf. Fig. 17a), serial longitudinal sections through the HN and third longitudinal tract layer. Five slow extensor linking segments (*a1*, *a2*, *p1*, *p2*, *e1*) occur here. A few dendrites are visible in the HN (arrows in *a*, *b*, and *c*). A branch from the integrative segment of *e1* (arrowheads in *a* and *b*) descends in M and ends (*) near its integrative segment. Dendrites from the other four slow extensors lie in the vertical G tracts (double arrows). *c*. Branches of *e1* in the curve of the HN are circled. Note longitudinal branch of *p1* (double arrows) along the VLT; *a, b* $\times 290$; *c* $\times 235$

In four of the most well-filled ganglia, e.g., Figure 3, groups of hair afferents projected into one or two glomeruli (Fig. 10). At most, eight glomeruli were filled in the ipsilateral arm of the HN. No glomerulus

contained a dense packet of filled processes; rather, a glomerulus was discernable because it contained a network of fine, ramifying processes. These processes delineated a distinct spherical region of the same size and shape as the glomeruli identified by Skinner ('85a,b). The glomerulus containing the greatest number of filled processes, and hence the one that appeared most distinctly, occurs in Figure 10. However, at this magnification ($\times 425$), an entire glomerulus cannot be photographed in one focal plane.

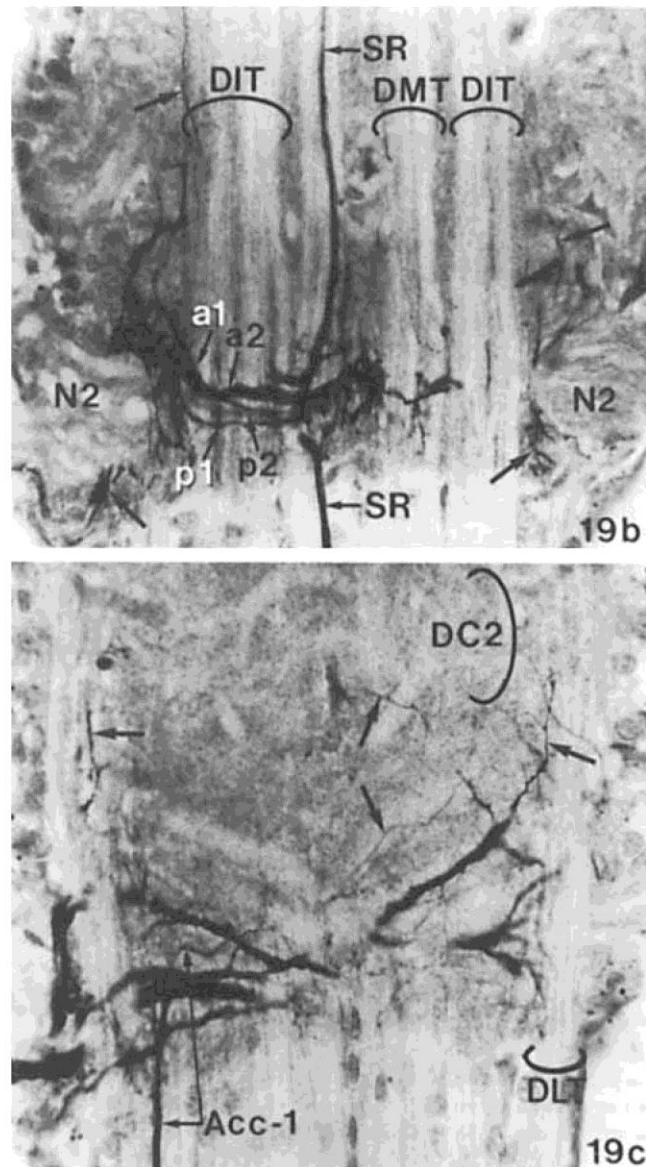


Fig. 19. G3 (cf Fig. 17a) with five filled slow extensor neurons. Serial longitudinal sections through the second commissure layer (a), second longitudinal tract layer (b), and first commissure layer (c). The integrative segment of e1 crosses in DC7 in (a) to exit ganglion in (c). It and the other slow extensors give off many dendrites (arrows) in the TN of the commissures and longitudinal tracts. SR axon in (a) bifurcates in DC4 in (b). Integrative segments of the anterior and posterior neurons cross the ganglion in DC4 in (b) and (c) with the most anterior one possibly in DC3 in (c). Branches of Acc-3, indicated by the longer arrow in (c). a $\times 185$, b $\times 235$, c $\times 270$.

Sensory hair axons, 1-10 μm in diameter, run rostrally in the nerve cord in three different longitudinal tracts: the ipsilateral and contralateral VITs and the ipsilateral VMT. In 37 ganglia, 77% of the axons that enter a ganglion have rostral axons extending to at least the next anterior ganglion. After entering the ganglionic core, most of the axons turn rostrally into the ipsilateral VIT and send branches into the arm of the adjacent HN (Figs. 5, 11). Axons running in the VMT often arise from branches in the curve of the HN, but in an SB1 fill, one axon also crossed through the anterior ventral commissure and extended into the ipsilateral VMT (Fig. 12). Some of the contralateral branches give off axon collaterals that extend rostrally in the VIT (Figs. 2, 8, 9b). After exiting from their ganglion of origin, all of the ascending axons (and collaterals) remain unbranched in the anterior connectives and adjacent ganglia (Fig. 13). The terminations of these sensory afferent axons were not found.

Most of the axons we filled were 4-10 μm in diameter. In only four ganglia did we fill any axons 1 μm or less in diameter (Fig. 14). These smaller axons ran in both the ipsilateral VIT and MIT. Some 2,000 axons are reported to run in N2 in *Orconectes virilis* (Somers and Nunnemacher, '70) and we assume a similar number occur in *P. leniusculus*. At most, we filled 17 larger diameter axons and 30-40 smaller axons in one ganglion, which is still a small fraction of the total number. Although we believe our sample typifies the afferent population, it is possible that we repeatedly sampled the same subset of afferents.

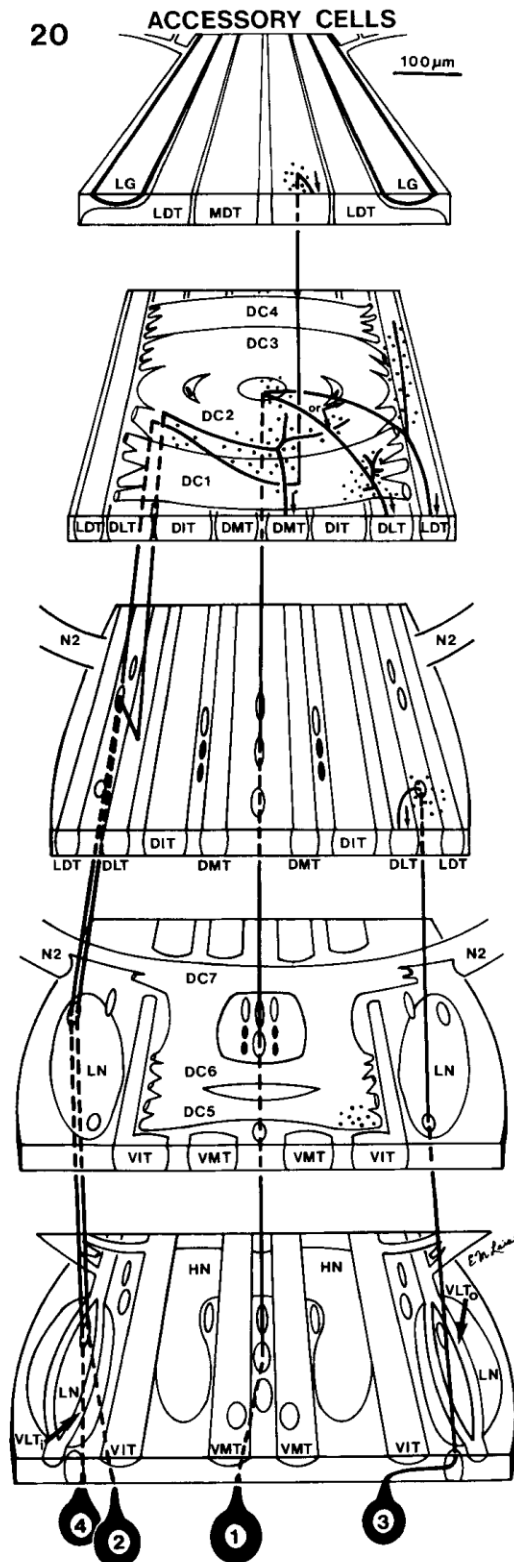


Fig. 20. Diagram of the routes taken by the four accessory cells through five layers of their abdominal ganglion of origin. Note alternate pathway for axon of Acc-1 and that the linking segments of Acc-2 and Acc-4 cross in the second tract layer. Acc-2 exits in the MDT, Acc-4 in the DMT. Rostral axons point towards reader (arrows). Stippling, regions of dendritic branching.

In no preparation did we find any sensory hair axons that extend caudally in the nerve cord. Only the SR axons (Figs. 4,15) extend in both directions from their home ganglia. Neurons in ganglia exposed to cobalt for long periods of time can leak cobalt as they die. Other unidentified neurons can incorporate the leaking ions, yielding preparations that appear to contain descending axons (Fig. 15). However, from sections to such ganglia it is clear that none of these caudal axons are continuous with any neurons in the ganglion of origin.

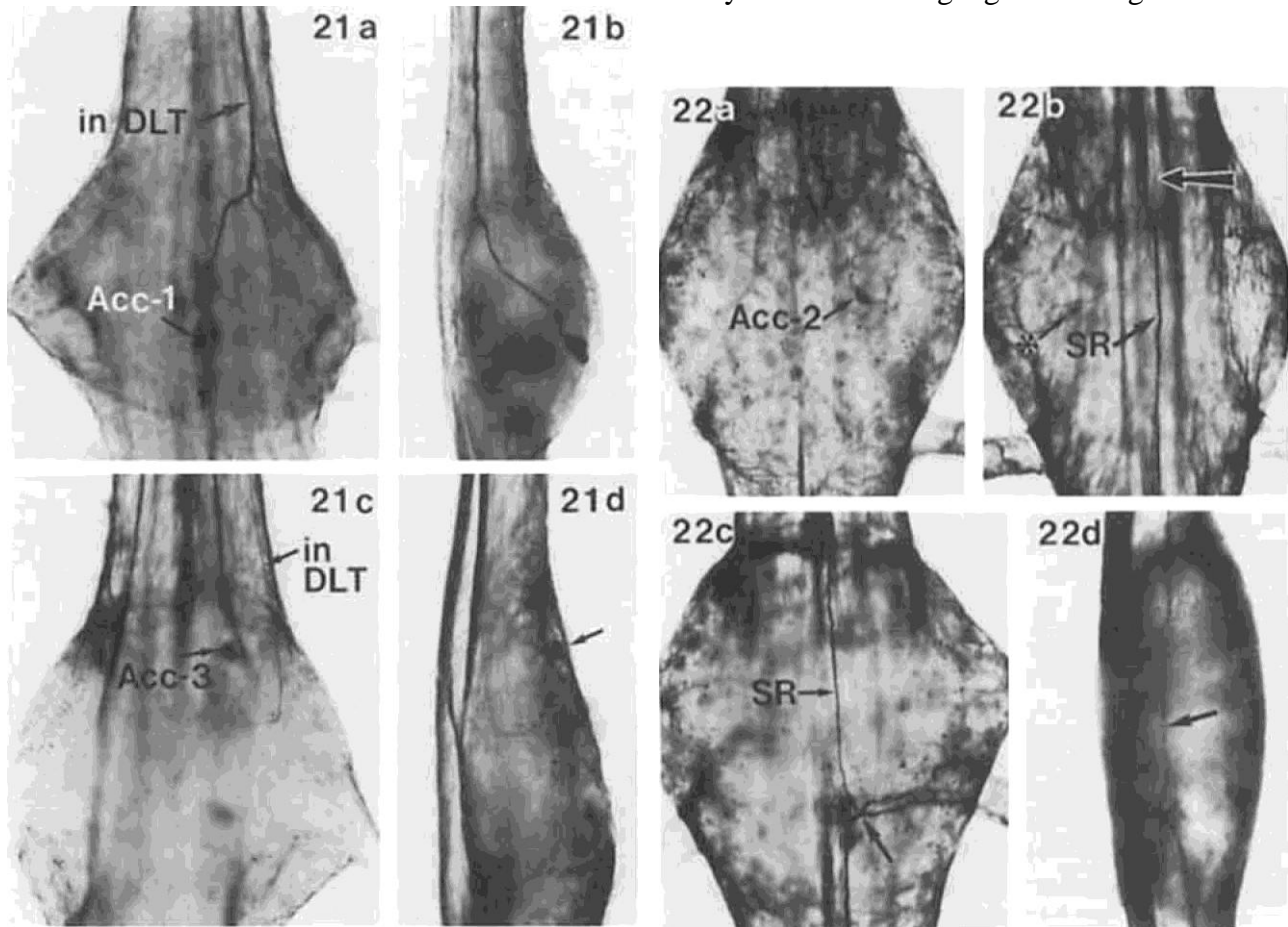


Fig. 21. Accessory cells that filled concurrently with fast extensor motor neurons. a and b. Dorsal and side views of Acc-1 in G5. Its axon lies in the DLT. c and d. Dorsal and side views of Acc-3 in another G5. Its axon is also in the DLT. Arrow points to soma in d. $\times 95$.

Fig. 22. a and b. G4, ventral and dorsal views of Acc-2 filled from G3. Note SR axon in b. Arrow points to Acc-2 axon. Asterisk indicates soma position. c. G3, dorsal view, two posterior slow extensors, one SR axon (note bifurcation at arrow) and Acc-2 axon are filled. d. G3, side view, note the rather central position of SR axon (arrow). $\times 95$.

Paths of the slow extensor motor neurons

We filled at most five slow extensor motor neurons through one N2 (Figs. 16, 17). Four cells occur in two ipsilateral clusters, one anterior (a) and one posterior (p). We arbitrarily designated the neurons in each cluster as number 1 or 2 (Fig. 16). The contralateral neuron is called e1, after Wine and Hagiwara's ('77) description. One or two stretch receptor (SR) axons from the muscle receptor organs (MROs) and one to four accessory cells to the MROs filled concurrently (Fig. 17) because their axons run in the same nerve. The paths of the SRs and accessory cells are described below in separate sections.

From their somata, the main neurites, or linking segments (Cohen, '70), of the five slow extensor motor neurons extend laterally toward the two outlying vertical G tracts (Figs. 17a, 18a) where they ascend through the ganglion. The linking segment of neuron p1 sends a few dendrites to the most ventral part of the HN. Similarly, the linking segments of the anterior neurons give off a few dendrites that lie at the junction of the HN and LN (Fig. 18a). Two branches from e1 descend from the integrative segment in DC7 (Figs. 16, 19a) through the HN and vertical tract M (Fig. 18c). The ventral portion of one of these branches from e1 crosses medially just below

the contralateral arm of the HN (Fig. 18a). This branch ends very close to its own linking segment in the contralateral tract G (Fig. 18b).

The integrative segments of the slow extensor neurons give off dendrites to the surrounding lateral neuropils (LNs) and send very fine ($< 0.5\ \mu\text{m}$ diameter) branches into the ipsilateral and contralateral HN. The posterior portion of the HN, just below the third tract layer, contains many dendrites, as do the tract neuropils (TNs) of the VITs and VLTs in the posterior half of the ganglionic core. In addition to branching in the posterior half of the LN, el sends a few dendrites into the TN of the adjacent contralateral VIT. Its descending branches, mentioned above, also give off dendrites to the curve of the HN (Fig. 18b,c) and into the TN of the ipsilateral VMT. Thus, most of the branches in the curve of the HN are from el. Neuron pl also sends a longitudinal branch along the ipsilateral VLT, (Fig. 18c) and dendrites into the TN of the ipsilateral VIT.

The second and first commissure layers contain many dendrites from the slow extensor neurons as well as the major branches that *cross* the ganglion. All of the integrative segments continue to rise in the vertical G tracts, to DC7 for el or to DC4 for the remaining four cells. In DC7, el crosses the ganglion to exit through the contralateral N2 (Figs. 16, 19a,b). This neuron sends many fine, longitudinal branches, $< 1\ \mu\text{m}$ in diameter, into the TN of the second layer of longitudinal tracts, in the posterior half of the ganglionic core. In particular, el branches in the contra-lateral LN and TN of the DLT (Fig. 19b). The anterior and posterior neurons bend towards N2 and cross the ganglionic core in DC4 of the first commissure layer, reaching the contralateral DLT (Figs. 16, 19b,c). These large branches, $5\text{--}7\ \mu\text{m}$ in diameter, send many smaller branches, $1\text{--}4\ \mu\text{m}$ in diameter, into the TN of DC3 and DC4, into the ipsilateral LN, and into the TN of the DLTs and DMTs (Fig. 19b,c). Dendrites from these cells also lie in the TN just posterior to the N2 roots (Fig. 19b). Only a few slow extensor dendrites extend above this level to reach the TN of the MDTs in the first longitudinal tract layer.

Paths of the accessory neurons

Three of the four accessory cells to the MROs are known to be feedback inhibitors of the stretch receptor neurons and the muscles of the MROs (Kutner and Eyzaguirre, '55; Eckert, '61; Jansen et al., '70a,b,c, '71a,b; Wine and Hagiwara, '77). The axons of these four motor neurons run in the n.r.m. with the slow extensor axons, but exit the nerve cord through the ganglion just anterior to the one in which their somata lie (Figs. 17d—f, 20–22) (Wine and Hagiwara, '77; Bastiani, '80). Interestingly, in each of the six preparations in which we successfully backfilled the *fast* extensor motor neurons, we concurrently filled one of the accessory cells. This is novel because the accessory cell axons are not known to run in the n.p.m., which innervates the fast extensor muscles. We filled accessory cell 1 (Acc-1) five times (Fig. 21a,b) and Acc-3 once (Fig. 21c,d). Acc-1 and Acc-3 branch in their ganglion of exit, but first we describe the branching patterns of the accessory neurons in their ganglion of origin.

The accessory cell somata are widely dispersed across the ganglion. Acc-1 (Figs. 17d,e, 21a,b), the main accessory (Wine and Hagiwara, '77), has a medial soma that can lie either contralaterally or ipsilaterally. The position of the soma, as we record it, may be caused by the shrinkage of the ganglion during silver intensification rather than by any developmental ambiguity. Its unbranched linking segment rises in vertical tract J (Figs. 20, 23a–c). When contra-lateral, Acc-1's linking segment crosses to the opposite hemiganglion shortly after leaving its soma. Acc-2 (Figs. 20, 22a,b) has a contralateral soma and its unbranched linking segment rises in the contralateral vertical tract G (Figs. 20, 24a,b). Acc-3 has an ipsilateral soma (Figs. 17d,e, 20, 21c,d) and its linking segment extends laterally to rise in vertical tract C (Figs. 20, 23a,b), at the anterior edge of the ganglionic core. Acc-4 (Figs. 17d,e, 20) has a very contralateral somat that appears at the level of the HN (Fig. 23b). Its linking segment rises in the contralateral vertical tract G (Fig. 23c–e). The linking segment of all of these neurons remain unbranched until the second commissure level.

None of the accessory cells support abundant branches in the ganglionic core. The main neurite of Acc-1 crosses through DC2 and DC1 from vertical tract J, to extend its axon either in the upper part of the ipsilateral DLT (Figs. 20, 23e) or in the LDT (Fig. 20; see also Fig. 25a,b). Its short integrative segment branches in the TN of DC1 and usually sends a branch caudally into the LDT at this same level. Acc-2 rises in its vertical tract

G until it reaches the first commissure layer, where it crosses to the ipsilateral side through DC2 and DC1 (Figs. 20, 24). Acc-2 also branches a few times in DC1 and sends its axon to the next anterior ganglion at a very dorsal level of the connective in the MDT (Figs. 20, 24d). Acc-3 gives off a few small branches into the TN of DC5 just before it extends its axon anteriorly into the ipsilateral DLT (Figs. 20, 23c). The integrative segment of Acc-4 heads medially toward the lower portion of the ipsilateral DMT from vertical tract G (Figs. 20, 23d-f). Its neurite crosses the midline in the top of DC2, branches in DC1 and DC2, and exits the ganglion in the ipsilateral DMT (Figs. 20, 23f). All of the accessory neurons run anteriorly to the next ganglion where they turn laterally in DC4 to exit in N2.

In their ganglion of exit, only Acc-1 and Acc-3 give off any branches. As it enters the ganglionic core (Fig. 19a) Acc-3 branches in the TN of DC7, posterior to the main branches of el. Acc-1 also branches in the posterior part of the ganglion, but in the more dorsal DC4 where its axon enters the ganglion. Acc-1 sends a large (5- μ m diameter) branch toward the ganglionic midline (Fig. 19c) where it branches, again forming a tuft of processes in DC4 and DC3 that occasionally reach DC2. As Wine and Hagiwara ('77) noted for *Procambarus clarkii*, the axons of Acc-2 and Acc-4 appear to lack branches in their ganglion of exit.

Paths of the stretch receptor axons

Two stretch receptor (SR) axons enter each hemiganglion through the posterior branch of N2 (Alexandrowicz, '51). The axons bifurcate ipsilateral to the midline (Figs. 19b, 22c, 26) in DC4. Each SR axon, approximately 5 μ m in diameter in our animals, extends for the length of the nerve cord (reviewed in Bastiani, '80). In the abdomen, these axons lie in the ipsilateral DMT (Figs. 19a,b, 23d,e, 24b, 27a,b). On occasion, an SR axon rises into the adjacent MDT in the core of each ganglion. Axons that do so return to the DMT in the connectives and give off their normal cohort of ipsilateral and contralateral branches.

Every SR axon gives off short side branches in each abdominal ganglion. These branches lie in the central TN between the DMTs, in both DMTs themselves, and in the TN of the ipsilateral DIT (Figs. 23c,d, 26, 27b). Contralateral branches occur in each ganglion. Branches can extend into both of the commissure layers (Figs. 23c, 27b) and can occur in the TN of DC5-DC7 in the second layer.

Wine ('77b) found that the synapses between SR axons and the fast extensor motor neurons were strongest in the ganglion of SR entry. To determine if the shapes of the SRs correlate with his physiological data, we quantified the amount of branching in each ganglion and obtained totals for G2-G5 in three nerve cords (Fig. 28). In each cord, one or two SR axons from G3 were filled. In all cases, G4, the ganglion that contains the accessory cell somata (see previous section), contains the greatest total branch length.

Paths of the fast extensor motor neurons

Like the slow extensor cell bodies, the somata of the fast extensor motor neurons are located in both hemiganglia. Their integrative segments cross the ganglion in the posterior commissures and their axons exit in the posterior branch of N2 (Figs. 29, 30). The five ipsilateral somata occur as a posterior cluster of four cells (P1-P4) and a single more anterior cell (A) (Fig. 30a). Three contralateral cells occur as a group of two anterior somata (C1 and C2) and a lone posterior neuron (C3). Most of the finer dendrites occur in the posterior two-thirds of the ganglionic core in the more dorsal tracts and commissures (Fig. 30b,d). The general characteristics of the fast extensor neurons are illustrated by two ganglia, each containing seven filled cells (Fig. 30).

The assignment of fast extensor neurites to particular vertical tracts is problematic. At the lower levels of the ganglia in these small (2-4 cm long) animals, the lateral vertical tracts E, G, and H, (Kendig, '67; Skinner, '85a) are difficult to define. In addition, the VLTs in these animals are displaced dorsally relative to their positions in the adults. The VLTs appear in the second commissure layer, not in the plane of the third longitudinal tract layer. We assign neurites to particular vertical tracts on the basis of the neurites' dorsal locations in these tracts. There is also some variability in a neuron's choice of vertical tract (Fig. 29). For example, P1 rises in tract E (Fig. 31b,g) or in tract M (Fig. 31c), and P3 rises in G (Fig. 31d,f) or in H (Fig. 31b).

With the occasional exception of P1, the fast extensor motor neurons, like the slow extensors, rise through the lateral vertical tracts. The linking segments of C1 and C2 ascend together in the contralateral vertical tract E to the first commissure layer (Fig. 31b-k). C3 sends its linking segment to the ipsilateral hemiganglion where it rises with P4 in vertical tract H (Fig. 31b,g-j). The linking segment of C3 crosses below the HN in the posterior ventral commissure (PVC) (Fig. 31a). This is our first evidence for the existence of the PVC in *R. leniusculus*. P1, P2, and P3 start to ascend near each other (Fig. 31b), but P3 eventually moves into tract G in the second commissure layer (Fig. 31e,f). P1 and P2 ascend in E anterior to P4 and C3 (Fig. 31b,g), but, as noted, P1 can also rise in M (Fig. 31c,f). Neuron A, if present, is the most anterior, ipsilaterally, and rises in E (Fig. 31c-e).

The lower levels of the ganglion are nearly devoid of dendrites (Fig. 31a,b), but a few fine dendrites ($\leq 0.5 \mu\text{m}$ in diameter) do occur in both arms of the HN and in the ipsilateral VMT. In ganglia containing neuron A, more dendrites are present at the lower levels. When P1 rises through the HN and vertical tract M, P1 branches there along with a dendrite from neuron A (Fig. 31d). These neurons are responsible for the few dendrites occurring in the HN and anterior ventral commissure (AVC) (Fig. 31c) at lower levels on the ipsilateral side. C1 and C2 also branch in the TN of their tract E and will continue to do so as they ascend (Fig. 31e—g). These branches may reach the LN (Fig. 31e) but never form a major arborization there. This is also true for branches in the TN of the ipsilateral vertical tracts G and E.

In the most dorsoal part of the HN, the amount of dendritic branching increases dramatically (Fig. 31e) and continues to be extensive through the first commissure layer (Figs. 29, 31j-1). Longitudinal dendrites run along the tracts in the second tract layer (Fig. 31e,f). In the second commissure layer, dendrites occur in the TN of the central portions of DC6 and DC7 (Fig. 31f,h,i) and many long contralateral branches exist (Fig. 31f,i). Dendrites extend into both LNs at the sides of the ganglion. The first commissure layer contains the major ganglionic crossings of the integrative segments of seven of eight of the fast extensors (Fig. 31j,k,l). Neuron A sends its integrative segment across the ganglion in DC7 to exit in N2 at a lower level than the other seven (Fig. 310). Dendrites from the dorsal integrative segments reach into DC2 in both hemiganglia (Fig. 31k) and occasionally into DC1. All of the integrative segments (including the one in DC7) reach the contralateral side. Dendrites continue into the upper part of DC2-DC4 in both hemiganglia, and many branches reach the MDTs and LDTs (Fig. 31m,n). Again, longitudinal branches occur in both hemiganglia in this dorsal layer of tracts (Fig. 311—n). None of the branches shows any contact with the giant axons.

DISCUSSION

The results presented here extend our knowledge of the partitioning of function in the crayfish abdominal ganglia. The three major neuropils are the most straightforward to consider. The HN is likely to be the major site for the integration of exteroceptive sensory input because it receives projections from primary sensory afferents. The HN receives input from hairs on the dorsal, ventral, and lateral (pleural) plates of the exoskeleton and probably from the swimmeret hairs (Leise, unpublished data). However, information from proprioceptors, i.e., the SRs, is not directly processed in the HN but is routed to the TN containing dendrites of the target motor neurons: the fast flexor inhibitor and slow and fast extensors (Eckert, '61; Wine, '77b; Wine and Hagiwara, '77). The two LNs are thought to contain the major components of the swimmeret pattern generators (Paul and Mulloney, '85; Skinner, '85a), their motor neuronal targets, and probably input from swimmeret sensory afferents (Leise, preliminary data).

The tract neuropils are more complicated issues, in part because the dendritic domains of neurons serving several motor acts overlap so widely (Fig. 32, Table 1). Excitatory command information for tail-flips, connections from the giant and nongiant axons to the fast flexor motor neurons, is probably integrated in the first tract and commissure layers (Leise et al., '86). Inhibition of the fast extensor neurons from these same sources (Wine, '77a) is also likely to occur there. Excitation of the accessory neurons, in their ganglion of origin, by the giant axons (Eckert, '61) is also likely to occur in the first tract and commissure layers. The tract neuropils of the second tract and commissure layers contain branches from all four of the motor neuronal populations we have examined (Fig. 32, Table 1; Leise et al., '86). As a result, the specific functional roles of these neuropils are likely to be complex. As more information is collected on the integrative characteristics and

ganglionic locations of the interneurons important to abdominal activities, our understanding of the functions of the tract neuropils will no doubt progress.

TABLE 1. Dendritic Projections of Accessory Cells, Stretch Receptor Neurons, Fast Extensor Neurons, and Slow Extensor Neurons Within the Ganglionic Neuropils^a

Accessories	Stretch receptors	Fast extensors	Slow extensors
—	—	LNs	LNs ^b
—	—	HN ^b	HN ^b
MDTs, LDTs	—	MDTs, LDTs	MDTs ^b
—	DMTs, DITs	DMTs, DITs, DLTs	DMTs, DITs, DLTs
—	—	VMTs ^b , VITs ^b , VLTs ^b	VMTs ^b , VITs, VLTs
DC1, DC2, DC5	DC1–DC7	DC1–DC4, DC6, DC7	DC3, DC4, DC7

^aDendrites in tracts and commissures are in TN.

^bRegions with only a few branches.

TABLE 2. Tracts and Commissures That Contain Major Branches of the Accessory Cells, Fast Extensor and Slow Extensor Neurons

	Accessories	Fast extensors	Slow extensors
Longitudinal tracts:	DMT, DLT, MDT, LDT	—	—
Vertical tracts:	C, G, J	G, E, H, M	G, M
Commissures:	DC1, DC2	DC3, DC4, DC7	DC3, DC4, DC7

Synaptic glomeruli and the horseshoe neuropil

A synaptic glomerulus is a distinctive synaptic complex formed by a spherical cluster of processes, morphologically distinguishable from the surrounding tissue and often set off by glial cells (reviews in Szentagothai, '70; Rakic, '77; Shepherd, '79). Glomeruli occur in the cerebellum, the olfactory bulb, the lateral geniculate body, and other thalamic nuclei of vertebrate brains (Szentagothai, '70; Rakic, '77; Shepherd, '79). In invertebrate brains they occur in cockroach antennal lobes (Chambille et al, '80; Ernst and Boeckh, '83) and in the accessory and olfactory lobes of lobsters and crayfish (Maynard, '71; Sandeman and Luff, '73). They also exist in crayfish abdominal ganglia (Skinner, '85b). Each type of glomerulus has its own distinctive structure, neuronal components, and integrative roles, but all glomeruli have certain features in common.

Sensory afferents of vertebrate and invertebrate glomeruli may innervate one or many glomeruli, depending on the afferent type (Szentagothai, '70; Ernst and Boeckh, '83). In each glomerulus of a vertebrate brain, the axon terminals of sensory afferents contact dendrites of relay or projection interneurons. Within a typical glomerulus, many small axon terminals or dendritic boutons converge on one large neuronal process that is either the pre- or postsynaptic cell. Local interneurons can also contact these neurons and mediate local inhibition (Szentagothai, '70; Rakic, '75). With the exception of the glomeruli in crayfish abdominal ganglia, the above mentioned invertebrate glomeruli are known to contain sensory afferent terminals as the presynaptic endings. Instead of one large axon terminal, as exists in vertebrate glomeruli, there are usually many axon branches. The output neurons are projection interneurons, but the local interneurons are often anaxonic (Ernst and Boeckh, '83). Synaptic glomeruli may represent an evolutionary convergence toward an efficient use of three-dimensional tissue space for processing sensory information.

As Skinner ('85a,b) suggested, the HN is the major target for axonal branches of the N2 sensory afferents. As in the deutocerebral neuropils of crayfish (Ernst and Boeckh, '83; Sandeman and Luff, '73), the sensory afferents innervate glomeruli within the HN. These HN glomeruli are 15–25 μm in diameter (Leise and Mulloney, '86) and are clusters of fine processes, mostly between 0.1 and 0.6 μm in diameter. Larger processes, 2–6 μm in diameter are associated with each glomerulus, usually toward the exterior of the mass (Skinner, '85b). In some glomeruli, large processes appear to be within the central confines of the glomerulus. As yet, we have no data to describe the receptive fields that are represented in the HN glomeruli nor the number of afferents that converge on the 20 to 30 glomeruli we estimate to be in each HN. An examination of Skinner's published and unpublished electron micrographs revealed that the large diameter processes bear both pre- and postsynaptic terminals. Because the axon terminals from the N2 afferents ramify as very narrow fibers throughout a glomerulus, we suggest that the large diameter process is a branch of a sensory interneuron that receives input from the afferents. In terms of their functional connections, the HN glomeruli may be similar to the pulvinar glomeruli of the thalamus (reviewed in Szentagothai, '70), in which thalamic sensory-motor axons converge onto a large branch of a relay interneuron. The HN glomeruli are not like the glomeruli in crayfish olfactory

lobes, which have no large diameter processes, nor like the accessory lobe glomeruli in which the large process is presynaptic.

Stretch receptor connections

That the SR axons from each segment extend the length of the nerve cord and branch in every ganglion (but the commissurals) was predicted by Eckert ('61) and confirmed by Bastiani ('80). Our findings differ from Bastiani's in certain respects. From his whole mounts, Bastiani reported seeing contralateral branches only occasionally. In our sectioned material, we saw contralateral branches from each axon in every ganglion. However, the ipsilateral branches are more numerous and physiologically more important; Wine and Hagiwara ('77) reported stronger ipsilateral synapses from the SR axons onto extensor neurons. SR axons branch between 3 and 25 times in any ganglion, with most ganglia containing between 10 and 25 branches. The number of branches in a ganglion parallel the data presented for total branch length (Fig. 28). We confirm what Wine and Hagiwara ('77) realized, that many of the finest branches cannot be resolved in whole mounts, but can be seen in sections.

The SR axons excite the accessory cells, are in turn reflexively inhibited by them, and excite the fast flexor inhibitor and fast and slow extensor motor neurons (Eckert, '61; Wine, '77b; Wine and Hagiwara, '77). The SRs overlap with their target flexor and extensor neurons in the second tract and commissure layers. Wine found that the PSPs between the SRs and the extensor motor neurons were strongest in the SR's ganglion of entry and that the responses of the motor neurons in more distant ganglia were relatively weaker. Thus, one might expect the SR axons to branch most profusely in their ganglion of entry. This was not the case. The total length of axonal branches was greatest in the next posterior ganglion, G4 (Fig. 28). G5 had the next greatest amount of axonal branches.

The SR branching patterns we found do not mirror the SR to extensor neuron synapses, but they may reflect the SR to accessory cell synapses. The accessory cells receive input from SRs in their own and in neighboring segments, but are most strongly activated by SRs in their own segment (Jansen et al., '70b, '71b). SR input to the accessory cells is presumably central, and for any abdominal segment occurs not in the ganglion of entry but in the next posterior ganglion. This is also the ganglion in which each segment's SR branches most extensively. The SRs connect to several different types of neurons (Wine, '77a,b; Wine and Hagiwara, '77), but we have found a correlation between the morphology and physiology for one of their most well described synaptic connections.

Role of contralateral afferent projections

The sensory afferent to sensory interneuron synapses in the terminal ganglion trigger one type of tail-flipping in crayfish. These synapses are presynaptically inhibited by other interneurons (Zucker, '72b; Kennedy et al., '74, '80; Bryan and Krasne, '77; reviewed in Wine, '84) and not by the contralateral afferents (Wiese et al., '76; Calabrese, '76a,b). The physiological evidence was reinforced by Calabrese's finding that none of the telson hair afferents he filled project to the contralateral hemiganglion, although a recent study of telson afferents demonstrates that the branches of some afferents cross into the opposite hemiganglion (Kondoh and Hisada, '87). The segmental hair afferents in N2 can also trigger tail-flips mediated by the lateral giant axons (LGs) (Zucker, '72a) or by the "nongiant" inter-neurons (Schrameck, '70; Zucker, '72b; Kramer and Krasne, '84). In 11 of 37 ganglia we found afferents with contralateral projections and axon collaterals. If the segmental afferents have physiological properties similar to those of the telson, it is likely that the contralateral projections will not be directly involved in presynaptic inhibition of the contra-lateral afferents. Sensory interneurons that have bilateral excitatory receptive fields exist in this animal (Calabrese, '76b; Sigvardt et al., '82). We suspect that the contralateral branches we found in N2 sensory afferents participate in this step of sensory integration.

Identification of slow and fast extensors

Six slow extensor motor neurons have been previously identified (Wine and Hagiwara, '77; Triestman and Remler, '75). These identifications were based on backfills of N2, on analogy with the six slow flexor motor neurons (Wine et al., '74; Leise et al., '86), and on physiological data (Fields and Kennedy, '65; Kennedy et al.,

'66; Wine and Hagiwara, '77). We found only five of the expected six neurons. We designate our contralateral neuron as e1, the slow extensor inhibitor, in accordance with Triestman and Remler ('75), Wine and Hagiwara ('77), and Miall and Larimer ('82). Neither we nor Wine and Hagiwara located an additional contralateral neuron, e5 (Triestman and Remler, '75).

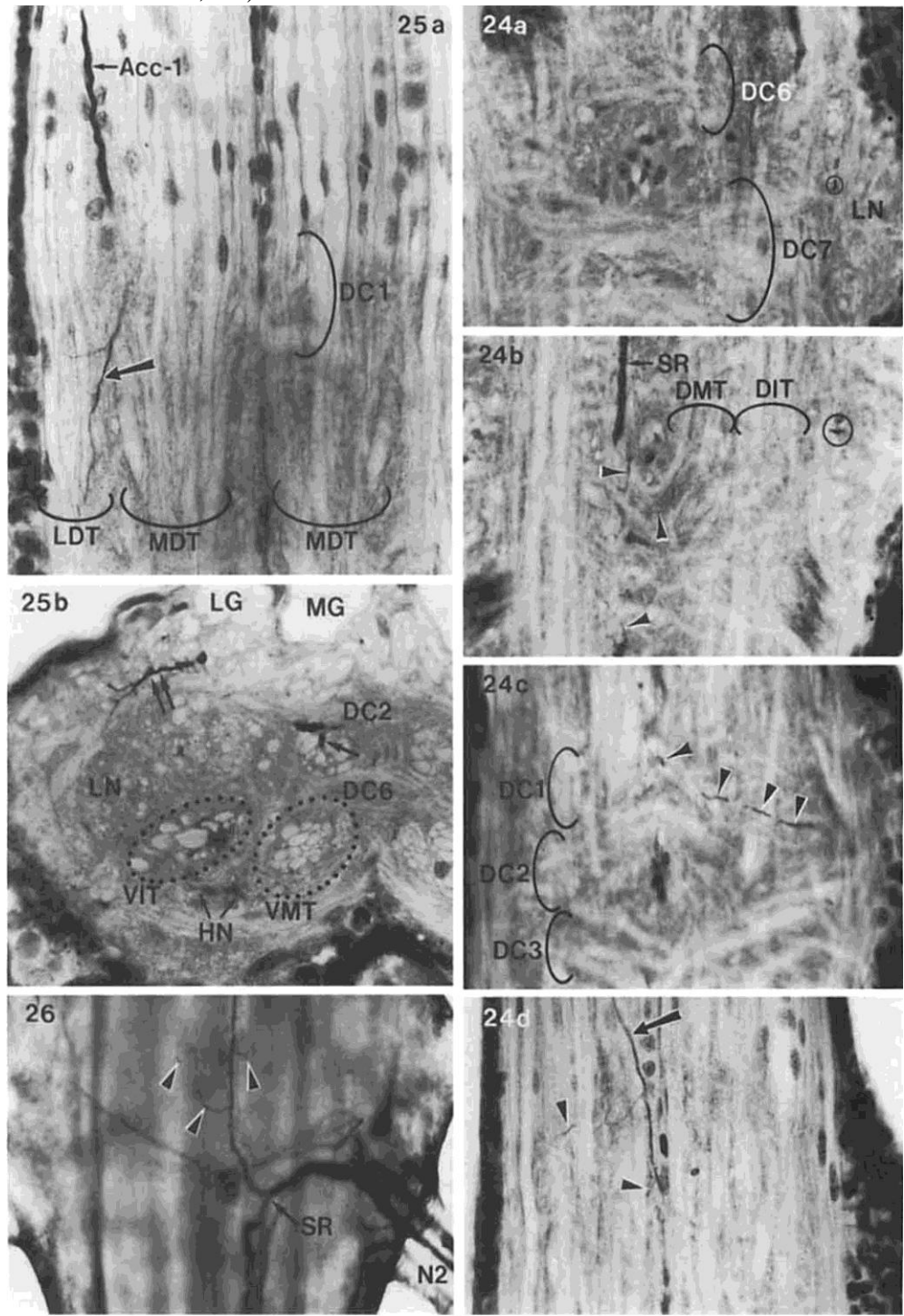


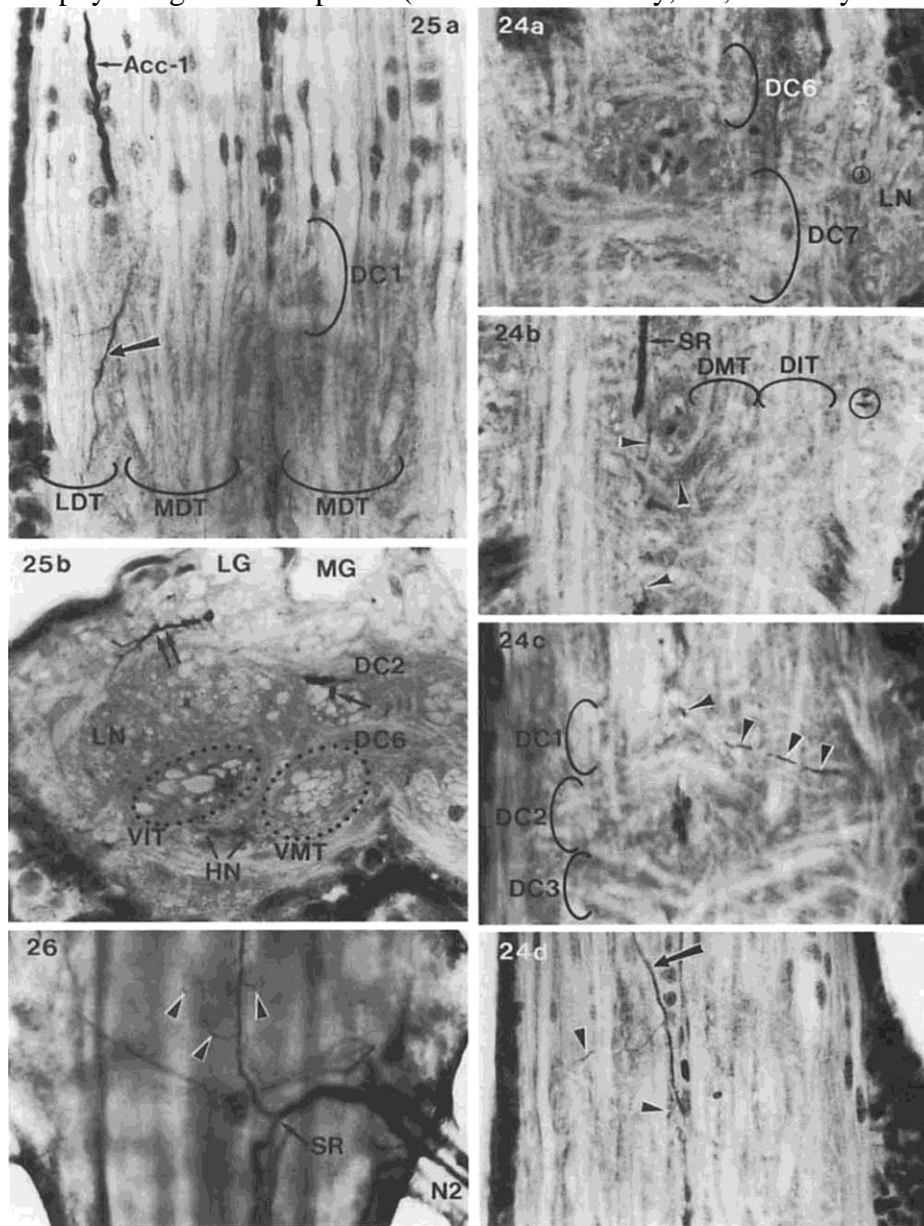
Figure 23

Fig. 23. G4, a series of longitudinal sections (cf Fig. 17d). The main neurites of three accessory cells, 1, 3, and 4, are identified in each section in which they appear. Only Acc-4 occurs in (0. a. Base of the ganglion where the HN and LNs are connected. Acc-3 rises in the ipsilateral vertical tract C and Ace-1 rises in tract J. $\times 310$. b. HN, LN, and third tract layer. Note lack of branches in ganglionic core. Asterisk is on Acc-4 soma. $\times 255$. c. Second commissure layer and parts of second longitudinal tract layer. Acc4 rises in the contralateral vertical tract G, Acc-3 extends its axon in the DLT. Note its few dendrites in the core (arrowheads). Medial branches (arrow) are from the SR axon. $\times 255$. d. Second longitudinal tract layer. SR axon is in DMT. $\times 220$.

e. First commissure layer and part of second tract layer. Axon of Acc-1 crosses over DC2 and DC1, branches in the core, then exits the ganglion in the DLT. Neurite of Acc-4 turns medially. SR axon lies in DMT. ×205.

1. First commissure layer, end of second longitudinal tract layer on the right side, and start of first longitudinal tract layer on the left side, beyond the DMT. Acc-4 (arrowhead) crosses over DC2 and exits in the ipsilateral DMT. ×205.

Wine and Hagiwara ('77) identified five small, anterior, ipsilateral somata as slow extensor motor neurons. Two of our somata (al and a2) lie in this group, but the other two (pl and p2) lie in the group of large, posterior ipsilateral cells they identified as fast extensor neurons. Wine and Hagiwara misidentified some of the extensor motor neurons because they filled the entire N2. Our previous analysis of the slow flexor motor neurons in *P. leniusculus* (Leise et al., '86) demonstrated neurons identical to those in *Procambarus clarkii* (Wine et al., '74), thus validating an inter-specific analysis. We have not attempted to correlate our four ipsilateral slow extensor neurons with the earlier physiological descriptions (Fields and Kennedy, '65; Kennedy et al., '66).



Figures 24-26

Fig. 24. *G4, serial longitudinal sections (cf. Fig. 22a) containing Acc-2 and an SR axon. Note lack of branches throughout most of the ganglionic core. a. Second commissure layer. Unbranched linking segment of Acc-2 (circled) is in vertical tract G. b. Second longitudinal tract layer. SR is in DMT and branches there (arrowheads). Acc-2 (circled) turns medially from tract G. c. First commissure layer, Acc-2 (arrowheads)*

crosses to medial MDT. d. Top of first commissure layer and first tract layer. Acc-2 branches in DC1 (arrowheads) and its axon (arrow) runs rostrally in the MDT. a,b ×235, c,d ×230.

Fig. 25. Two different G4s, each containing Acc-1 backfilled from G3 along with fast extensor motor neurons. a. Horizontal section through the junction of the first tract and commissure layers. The axon of Acc-1 and one of its characteristic branches (arrow) both lie in the LDT. × 300 b. Transverse section just anterior to the central artery, left hemiganglion. Axon of Acc-1 (arrow) lies in the DMT, in the connective it moves to the DLT. The integrative segment just above the axon is in DC2. The large branch (double arrows) is in the LDT. Dark areas in the VIT and HN are intensification artifacts. × 350.

Fig. 26. G3, dorsal view of SR axon, labeled at bifurcation. Note the ipsilateral branches and typically long contralateral branches (arrowheads). Two lightly filled slow extensors are out of focus below. × 110.

Wine and Hagiwara ('77) show that none of the extensors contact the giant axons. Our findings confirm this observation. They also described crossings in dorsal posterior commissures that we have elaborated in more detail. Wine and Hagiwara describe the branches of these neurons as being predominantly ipsilateral; we found many narrow dendrites in both hemiganglia.

We filled eight fast extensor motor neurons, although at most seven appeared in any one ganglion. Parnas and Atwood ('66) describe five excitatory fast extensor motor neurons and one inhibitor in a crayfish. Otsuka et al. ('67) identified three somata of ipsilateral fast extensor neurons, and one contralateral inhibitor in abdominal ganglia 1-4 in a lobster. Wine and Hagiwara ('77) also identified a large contralateral soma as the fast extensor inhibitor and a caudal cluster of five or six fast extensor exciters. Of our three contralateral fast extensors, we cannot suggest which might be the inhibitor. Our ipsilateral caudal cluster of four fast extensor neurons overlaps with Wine and Hagiwara's caudal cluster. Our anterior fast extensor is one of their misidentified slow extensor neurons.

Accessory neurons

In our fills of the n.r.m., we identified the same four accessory neurons as occur in *Procambarus clarkii* (Bastiani, '80; Wine and Hagiwara, '77). Only three have been identified physiologically (Jansen et al., '70a,b,c,'71a,b; review in Wine and Hagiwara, '77). We did not observe the profuse branching in the ganglion of origin recorded by Bastiani. This may result from our using a different species or from our use of very small animals.

Accessory cell axons are known to lie in the n.r.m. with the axons of the slow extensor motor neurons. Curiously, in our fills of *fast* extensor neurons from the n.p.m., we routinely filled Acc-1 in the adjacent posterior ganglion, but did not fill Acc-2 or Acc-4, the SR axons, or the slow extensor neurons. The MRO accessory cell axons are not known to send a branch in the n.p.m. to innervate the fast extensor muscles. Given that no systematic error in technique caused an accessory cell to fill with the fast extensors, we suggest that Acc-1 may be a common inhibitor, inhibiting the fast extensor muscles as well as the MROs. The physiological interactions of Acc-1 with the fast extensor muscles merit careful attention. When filling extensor motor neurons, Wine and Hagiwara ('77) and Bastiani ('80) used the entire N2. Thus, their results cannot be brought to bear on this question.

Dendritic domains of flexor and extensor motor neurons

In terms of the commissures and tracts in which major neurites lie (Table 2) and the locations and extent of their dendritic domains (Table 1), the fast extensor neurons are more like the slow extensors, with the exception of the greater amount of branching in the HN and LN by the slow extensors (Fig. 32), than like either of the flexor neuron populations. As Wine ('77b) pointed out, the flexor and extensor neuronal systems are not symmetrically antagonistic sets of neurons. For example, the SRs do not contact the fast flexor excitor neurons, but do contact the fast extensor neurons. The dendritic domains of the slow and fast extensor neurons are also more alike than those of the two types of flexor neurons (Fig. 32; Leise et al., '86). The slow flexor neurons have the most widespread dendritic arborizations of the four motor neuronal groups (Leise et al., '86).

Phasic extension of the crayfish abdomen can be elicited by waterborne stimuli, as long as the mechanoreceptive hairs of the abdomen remain intact (Reichert et al., '81; Wine and Hagiwara, '81). Phasic flexion cannot be elicited in the same manner. As anticipated, the central projections of these neurons suggest that phasic extension could be reflexive; both the mechanoreceptors and fast extensor neurons branch in the HN. The fast flexor neurons bypass this neuropil entirely (Leise et al., '86).

During bouts of backward swimming by tail-flipping, the fast extensor neurons can be excited without sensory feedback from flexion (Reichert et al., '81). This supports the idea that nongiant extension is driven centrally. The branching of the extensor neurons in the dorsal tracts suggests that input from the dorsal cord axons or corollary discharge axons occurs in this region. Thus, the central projections of the fast extensor neurons support the physiological evidence for their being activated by two separate systems (Reichert et al., '81).

Organization of axons within the nerve cord and ganglia

The positions of the three types of intersegmental neurons encountered in this study—sensory hair afferents, stretch receptor neurons, and accessory cells—are diagrammed in Figure 33. Sensory afferents run rostrally in both the VITs and VMTs, with the larger axons, $> 4 \mu\text{m}$ diameter, in the VITs and the small diameter axons, $< 1 \mu\text{m}$, in both the VITs and VMTs. Three-quarters of all the sensory afferents that enter a ganglion send axons rostrally in the nerve cord. None send them caudally. This suggests to us that our sensory afferents may comprise at least two populations, one with and one without rostral axons. Both hair afferents and cutaneous mechanoreceptors (CMs) (Pabst and Kennedy, '67) have axons in the anterior branch of N2. These two types of receptors, with their two different functions (the CMs inhibit the tonic flexors and swimmeret motor neurons, causing slow abdominal extension and relaxation of the swimmerets) might be the sources of the two morphologically distinct sets of afferents. Alternatively, the two populations of afferents may simply stem from the variability of our success at filling cells.

The locations of the primary sensory afferents in Wiersma areas 83, 84, and 85 overlap with the positions of the VMTs in the connectives (Fig. 33; Wiersma and Bush, '63). The bundle of contralateral sensory axons (Fig. 33a) does not resemble any we found.

In a few fills of the anterior branch of N2 made with long filling times, we found what appear to be descending primary sensory afferents (Fig. 15). From the sections we determined that these axons were not N2 sensory afferents because they were not connected to axons in the filled nerve. Hughes and Wiersma ('60), Wiersma and Hughes ('61), Wiersma and Bush ('63), Calabrese ('76a,b), and Bastiani ('80) identified both ascending and descending primary mechanosensory exteroceptors from N2, although only Bastiani published data from filled neurons. Bastiani's fills were made from the entire N2, filled for 24 hours, and he reported that the interior of the home ganglion was obscured by cobalt leakage. Our results suggest that the reports of descending primary sensory axons (with the exception of the SR axons) from N2 were erroneous.

In the abdomen of crayfish, SR axons lie just ipsilateral to the midline, in the DMTs, not directly below the giant axons (Bastiani, '80). These SR axons evoke extension by exciting the flexor inhibitor and the fast or slow extensor neurons (Eckert, '61; Wine, '77b; Wine and Hagiwara, '77). These SRs are among the ones described by Wiersma and Hughes ('61) and Wiersma and Bush ('63) (Fig. 33).

Comparison with the organization of insect ganglia

The array of longitudinal tracts in crayfish abdominal ganglia was named after that in orthopteran insects because of their striking morphological similarity (Skinner, '85a,b). Intersegmental sensory afferents from wind-sensitive hairs on the locust head and thorax run in the MVT (Anderson, '85). We expected the axons of sensory hair afferents to run in the homologous tracts in crayfish. On first examination they do not. The sensory axons in *R. leniusculus* lie in the VMTs and VITs. We have been unable to locate any MVTs or LVTs, the fourth tract layer (Skinner, '85a) in this species. We suspect that the MVTs and LVTs have become fused with the third tract layer. Until the routes of sensory axons are studied in *Procambarus clarkii* or other crayfish with a fourth tract layer, we will not know if *R. leniusculus* is the exception or the rule. Pending further study of afferents in

other crayfish, we suggest that the intersegmental sensory hair afferents in insects and crayfish lie in homologous tracts.

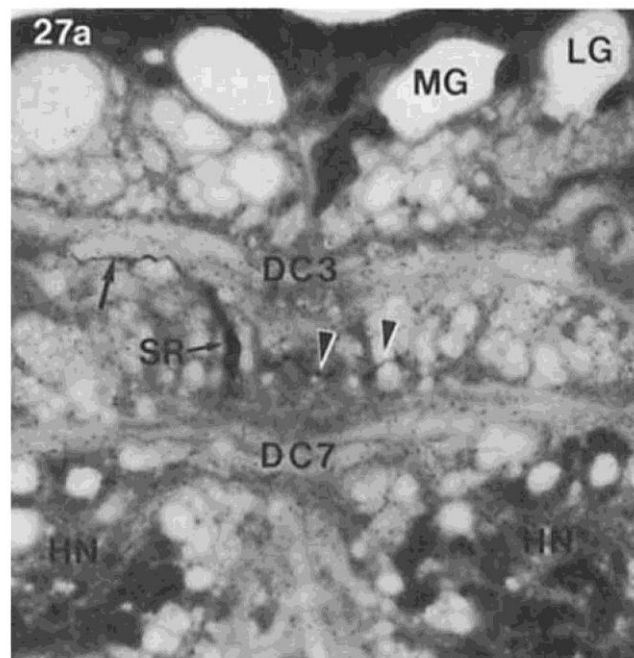
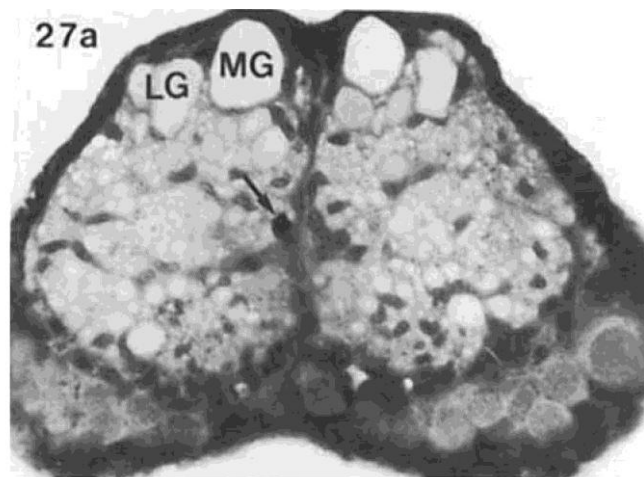


Fig. 27. G5, transverse. a. Anterior connective. SR axon (arrow) lies in the DMT. $\times 280$. b. Posterior ganglionic core. Note branches in contralateral DMT (arrowheads). Ipsilateral branch (arrow) runs along DC3. $\times 475$.

Historically, the ventral portions of insect ganglia were thought to be devoted to sensory processing (reviews in Pipa et al., '59; Murphey et al., '85). However, dorsal and intermediate level neuropils also receive afferent input, albeit from different populations of receptors. The ventral neuropil in crickets receives input from cercal, abdominal, and thoracic bristles, and in locusts, from hairs of the thorax and leg (reviews in Murphey et al., '85; Hustert et al., '81). The ventral neuropil, or ventral association center (VAC), has been subdivided into several regions: the bristle or ventralmost VAC (BN or vVAC), the VAC, and the medioventral VAC (MVAC). The cricket vVAC receives afferents from cercal bristles (Johnson and Murphy, '85; Murphey et al., '85) and the locust vVAC, from single tactile thoracic hairs (Pflüger et al., '81). The VAC receives afferent projections from thoracic hairs in locusts (Pflüger et al., '81) and bristles of the wings or thorax in crickets (Johnson and Murphey, '85). The MVAC receives input from internal proprioceptors (Braunig et al., '81). Because both the VAC and vVAC receive input from hairs distributed over the body surface, it may not be useful to separate these areas for our purposes. The crayfish HN receives input from external mechanoreceptors and is thus the homologue of the joint vVAC-VAC neuropil. Until projections from the swimmeret or thoracic chordotonal organs are examined, we cannot know which area in the crayfish neuropils is homologous to the MVAC.

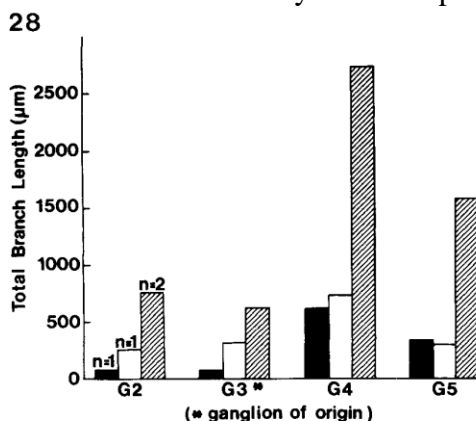


Fig. 28. Total length of axonal branches from SR axons in four abdominal ganglia. G3 was ganglion of origin in each nerve cord. n is number of filled SR axons. Black, white, and hatched bars identify individual nerve cords.

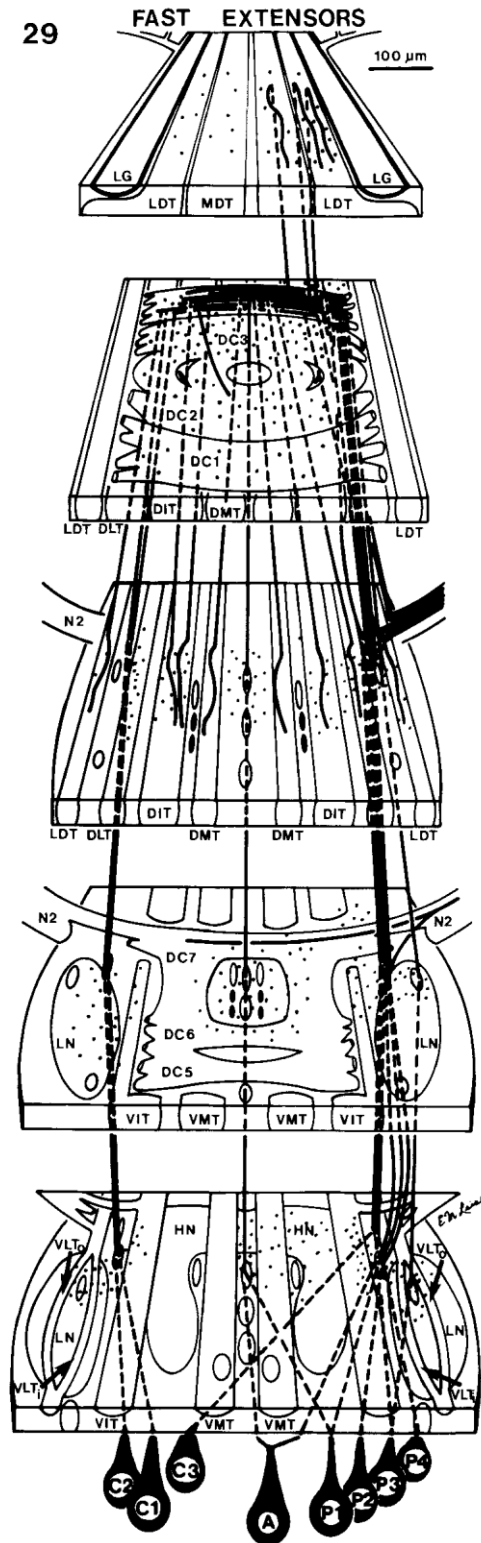


Fig. 29. Diagram of the routes taken by the major neurites (solid and dashed lines) of eight fast extensor motor neurons in five levels of an abdominal ganglion. Note alternate pathways observed for neuron P1. P4 has also been observed in vertical tract H rather than G as diagrammed here. Several neurites can also cross the ganglion in DC3 rather than in DC4 as shown. Stippling, areas of fine dendritic arborization.

The four thoracic SRs in locusts have much more complicated branching patterns than the crayfish abdominal SR axons, but they too run in the ipsilateral DMTs and MDTs. The insect SR axons also branch profusely in these tracts (Altman and Tyrer, '77; Tyrer and Altman, '74; Tyrer and Gregory, '82). Although the descriptions of the SR axons in locusts made by Altman and Tyrer do not place them in identified tracts, it appears from

their figures that the main posterior medial branches run in the MDT and then move to the DMT, whereas the posterior medial lateral branches run in the MDT and LDT. The medial anterior branches also run in the DMT and in a dorsal commissure. Thus, it seems that the SR axons are also evolutionarily conservative in their choice of longitudinal tracts. The profuse branching of the locust SR is expected, as these neurons are involved in several functions, such as control of wing beat frequency and motor neuronal phase relationships (Altman and Tyrer, '77), whereas those of the crayfish have a more limited behavioral role.

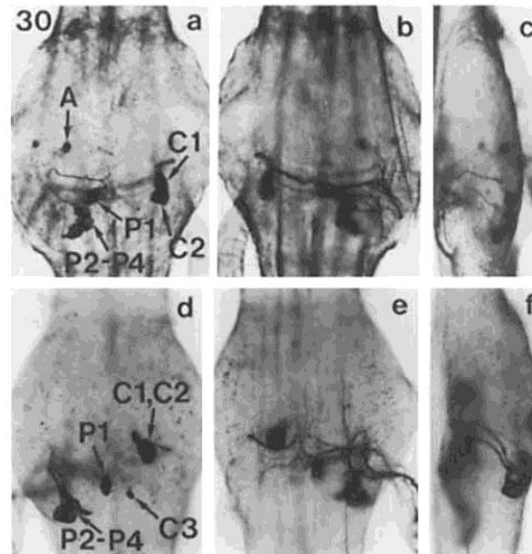


Fig. 30. Wholemounts of two ganglia, each with seven backfilled fast extensor motor neurons. a-c. G4 in ventral, dorsal, and side views. Side view shows five ipsilateral neurons. d-f. G3 in ventral, dorsal, and side views. a,b $\times 100$, c,d,e,f $\times 65$.

Somatotopic organization in the crayfish neuropils?

Several types of invertebrate sensory neuropils show somatotopic organization of the afferent axon terminals (Romer, '83; Johnson and Murphey, '85; Strausfeld, '79; Wohlers and Huber, '85; Murphey, '85; Levine et al., '85) as do various areas in the vertebrate cortex (reviewed in Shepherd, '79). Such representation of bodily receptive fields as orderly arrays of sensory neurons have not yet been discovered in the crayfish HN.

Topographical order for body musculature occurs in the mammalian spinal cord (review in Shepherd, '79) but is poorly known in vertebrate and invertebrate brains. Levine and Truman ('85) found that somatotopic mapping of dendrites from abdominal motor neuron occurs in the moth *Manduca sexta*. In this animal, motor neurons to lateral muscles have lateralized dendritic trees, but those serving muscles near the midline have bilateral dendritic projections. Levine and Truman found no anterior to posterior organization, although Tyrer and Altman ('74) noted that the dendritic trees of motor neurons innervating more posterior locust flight muscles were more dorsal than those to anterior muscles. In the neuropils containing crayfish flexor (Leise et al., '86) and extensor motor neurons, we found no somatotopic arrangement, but until a series of individually filled neurons is examined, somatotopic organization cannot be eliminated. Velez and Wyman ('78a,b) described the orderly innervation patterns of the slow flexor muscle fibers and proposed several hypotheses to account for this situation. The layout of these muscles in a flat sheet makes them a most useful system in which to look for systematic dendritic domains.

Conclusion

Our results support Altman's ('81) hypothesis that different neuropils are responsible for the various motor and sensory roles played by a ganglion. Discrete neuropils may be most easily recognized in ganglia $\geq 500 \mu\text{m}$ in diameter, that produce complex motor activities requiring the coordination of numerous muscles, or that integrate information from a large and/or diverse field of sensory receptors. In addition, our findings suggest that the nervous system of the two major arthropod groups, the Crustacea and Uniramia (which includes the Onychophora, Myriapoda, and Hexapoda), are built around the same basic framework. This uniformity in neural architecture calls into question the proposal of Manton and Anderson ('79) to erect the Crustacea and Uniramia as discrete arthropodan phyla. Even insects as small and advanced as *Drosophila*, where one might

expect a great deal of miniaturization and specialization to have taken place, have nervous systems that are arranged like those of the larger grasshoppers and crayfish (Thomas et al., '84). Thus, even the advanced insects have retained neuroanatomical features that may be basic to the arthropod plan of organization. It yet remains to be determined how widespread this neural foundation is among the other arthropods, but it is clearly flexible enough to allow for the widely differing behavioral requirements of these two major groups.

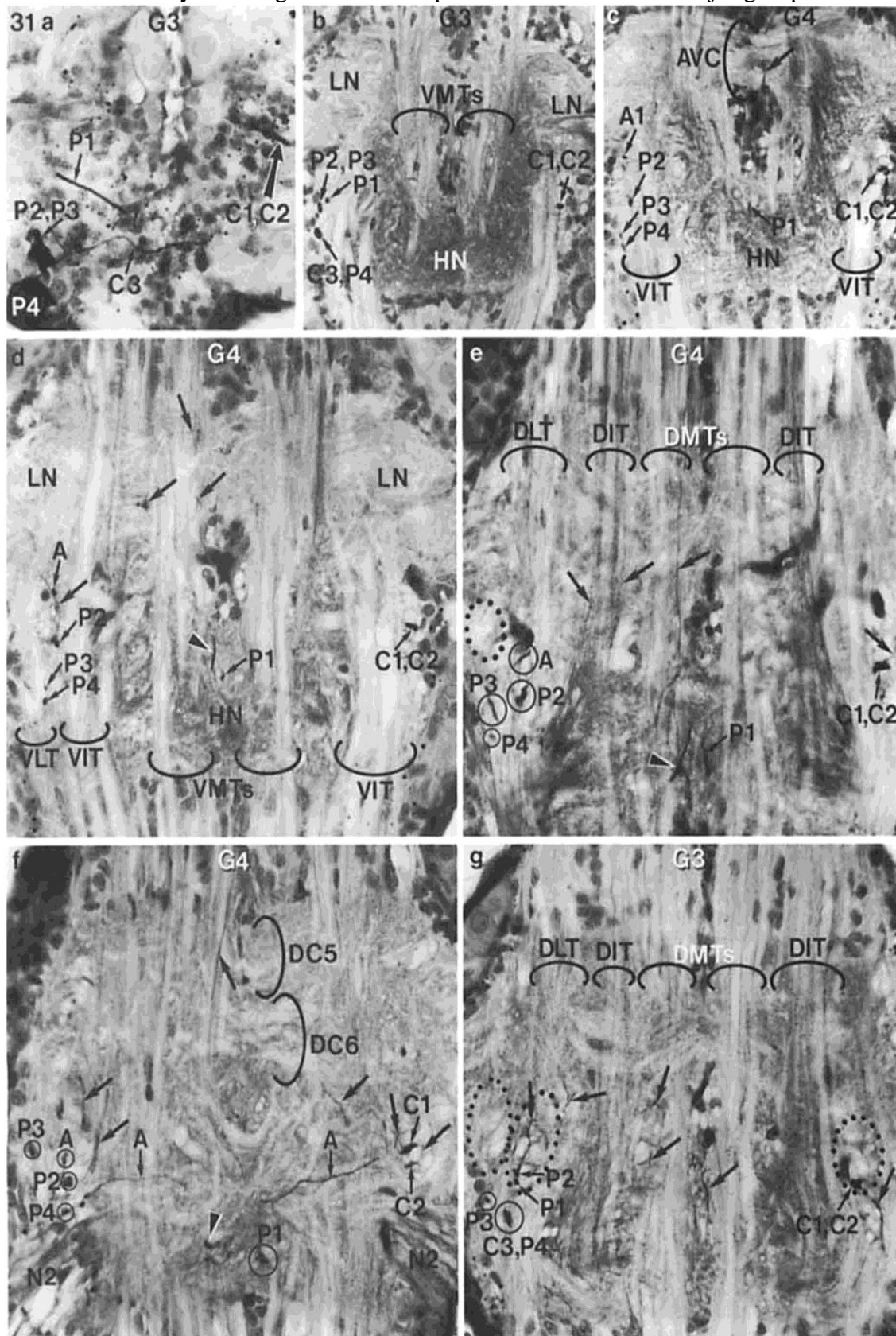


Figure 31

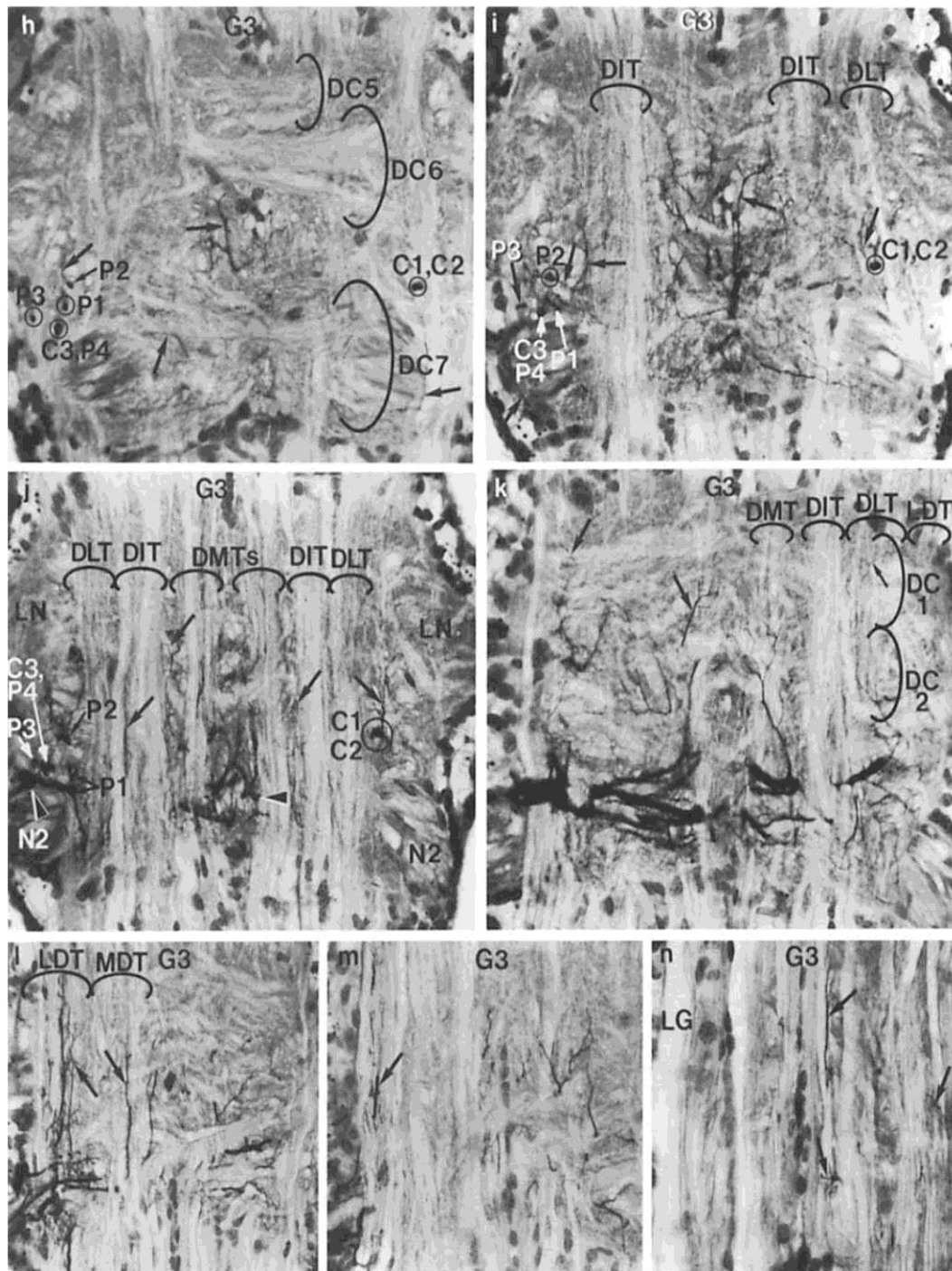


Figure 31 continued

Fig. 31. A series of sections through two ganglia, each with seven filled fast extensor neurons. *a, b, g-n.* G3 (cf. Fig. 30 d-f). *c-f.* G4 (cf. Fig. 30 a-c). Linking and integrative segments labeled. Dendrites at arrows. *a.* C3 crosses through PVC below the HN. $\times 175$. *b-d.* sections through HN and third tract layer. *e, g, j.* Second tract layer. *f, h, i.* Second commissure layer and part of second tract layer. *k.* First commissure layer and second tract layer. *l* and *m.* First commissure and first tract layer. *it.* First tract layer. *b.* C1 and C2 rise in vertical tract E. Ipsilateral tracts E and H appear as one tract containing P1-P4 and C3. $\times 140$. *c.* A and P2 rise in tract E, P3 and P4 in tract H. Note dendrite in AVC. $\times 160$. *d.* The main neurite of P1 and a branch from neuron A (arrowhead, also in *e* and *f*) descend in the curve of the HN. Note dendrites in TN of E. $\times 215$. *e.* Longitudinal dendrites occur in the TN of the DMT, DIT, and in contralateral tract E. P3 moves toward tract G (dotted circle). $\times 215$. *f.* Neuron A crosses in DC7. Dendrites occur in TN of DC6, DC7, and the remains of the DMT and DITs. $\times 205$. *g.* Note alternate route of P1, in vertical tract E with P2. Also note dendrites in this vertical tract, in the contralateral tract H and in the TN between the DMTs. Locations of

ipsilateral tracts G and E and contralateral tract E, dotted circles. Note P3 is closer to tract H here. $\times 205$. h. Note dendrites in TN of DC6 and DC7. $\times 200$. i. Note dendrites in TN of commissures between DITs and near lateral vertical tracts. $\times 190$. j. Most dendrites occur laterally or in the TN between the longitudinal tracts. Note parts of integrative segments (arrowheads) in left N2 and in the bottom of DC4. $\times 190$. k. The integrative segments of seven of the fast extensors cross the ganglion in DC3 and DC4. Dendrites extend into DC1. $\times 120$. l, m, n. Dendrites extend into the MDTs and LDTs ipsilaterally (l and m) and contralaterally (n). $\times 155, 180, 220$

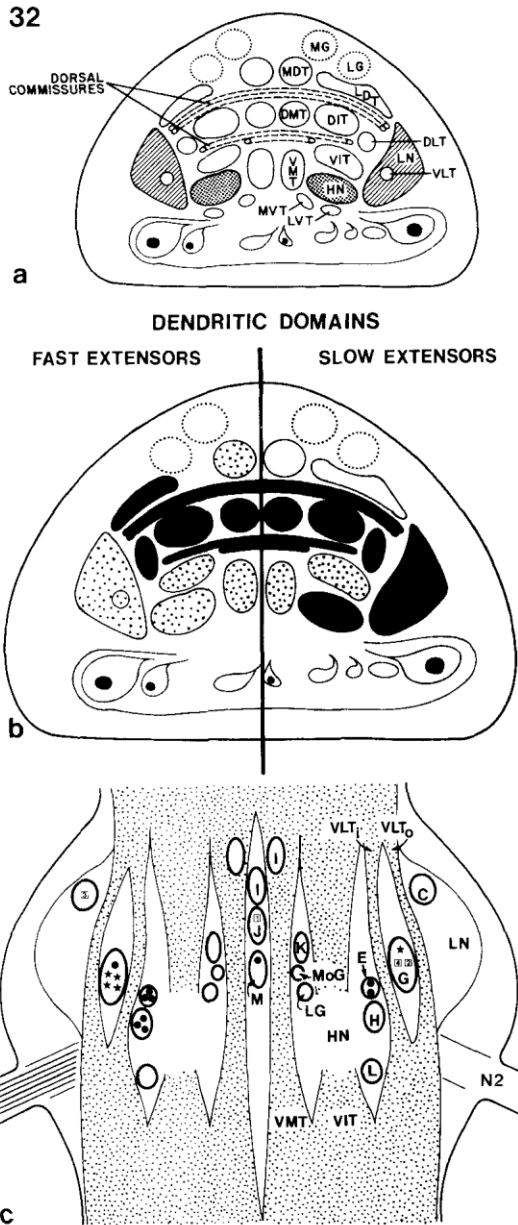


Fig. 32. a. Diagrammatic transverse section through the middle of an abdominal ganglion with commissures, tracts (solid lines), and neuropils (hatching) labeled. b. Transverse section as in a, demonstrating the dendritic domains of fast and slow extensors. Stippled areas contain relatively fewer dendrites. c. Diagrammatic longitudinal section through the third tract layer showing locations of accessory cells (numbered boxes), slow extensor (*), and fast extensor (●) linking segments drawn as if the left N2 were backfilled. Size of the vertical tracts is exaggerated and axonal positions within them are purely illustrative (modified from Skinner, '85a).

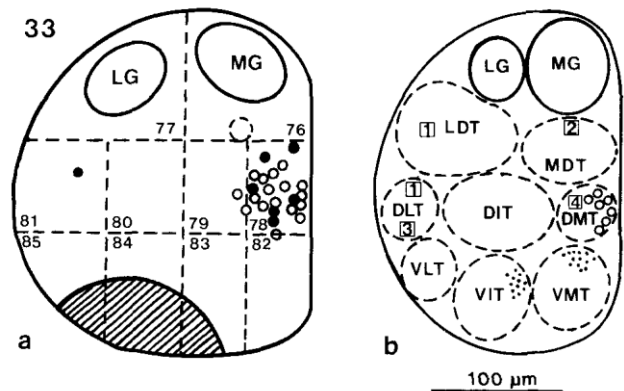


Fig. 33. Comparison of the locations of sensory afferent axons within the connectives of the abdominal nerve cord as described by earlier workers and by us. a. Diagram of the right abdominal connective of G3-G4 of *Procambarus clarkii*, demonstrating the locations of ipsilateral hair afferents (hatched area); contralateral afferents (dashed circle); SR axons from the MROs responding to flexion (○); and SR axons responding to extension (●). These latter axons probably respond to stretching of the nerve cord (Wiersma and Bush, '63). This diagram contains a composite of axons located by Wiersma and Bush ('63) and Wiersma and Hughes ('61). Labeled areas are according to Wiersma and Hughes ('61). b. Diagram of similar connective from *P. leniusculus*. SR axons as in a. Boxed numbers are accessory cell axons; dots are sensory hair afferents.

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